DET KONGELIGE INDUSTRI-, HÅNDVERK-
OG SKIPSFARTSDEPARTEMENT

NORSK POLARINSTITUTT
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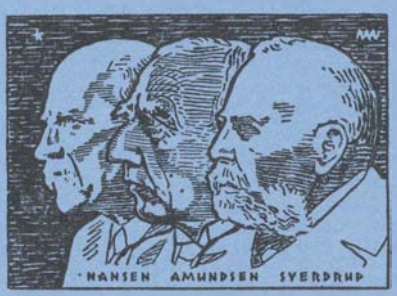
Nr. 95

HYPERVITAMINOSIS A

A STUDY OF THE EFFECT OF EXCESS OF VITAMIN A IN EXPERIMENTAL ANIMALS

BY
KÅRE RODAHL



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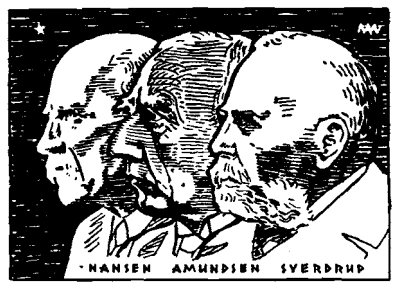

OSLO
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1950

A. W. BRØGGERS BOKTRYKKERI A/S

To my wife

Contents.

Preface.

The present work is a continuation of a previous study by the author of the toxic effect of polar bear liver. It has been carried out at the Institute of Physiology, Oslo University, during the period October 1947 to February 1949. I am greatly indebted to the Head of this institute, Professor dr. med. Einar Langfeldt, for placing the necessary laboratory facilities at my disposal.

The work has been carried out during a full-time grant from Norsk Polarinstitutt, for which I express my sincere thanks. My thanks are also due to the Head of that institute, Professor dr. phil. H. U. Sverdrup, for his interest and help in the completion of this work.

Technical assistance has kindly been given by Mr. E. Kvalheim, Mrs. Inger-Johanne Ingebrigtsen, Mr. J. Bøe and Miss Ellen-Margrethe Berg, to whom I extend my sincere thanks. I am also indebted to Dr. F. Clausen for roentgenological assistance, — to Mr. P. Scheffer Dahl, Dr. V. L. Segalstad and Dr. K. Heiberg for assistance with microphotography, — and to all my other colleagues who have rendered me help in various ways during this work.

Institute of Physiology, Oslo, August 1949.

Kåre Rodahl.

I. Introduction.

1. Hypervitaminosis A in Experimental Animals.

The condition of hypervitaminosis A was first described by Takahashi, Nakamiya, Kawakimi and Kitasato (1925) who, testing the effect of excess doses of their crude vitamin A concentrate "Biosterin" when given orally to rats and mice, observed loss of hair, emaciation, and paralysis of the hind legs. The animals died after periods varying from a few days to several weeks, and by postmortem examination, fatty degeneration of the liver, kidney and heart, was found, as well as hyperemia and sometimes hemorrhage in the intestines and lungs.

Several workers, including Takahashi and collaborators, and Matsuoka (1934), found that excess of vitamin A concentrate was also toxic when injected, usually causing death with cramp in less than one hour after the injection, while Moore and Wang (1945), who injected massive doses of vitamin A either subcutaneously or by intraperitoneal injection, found that the reaction never amounted to anything more than a temporary cramping and twitching of the muscles of the hind legs. In no instance in their experiment did an animal die as a result of this treatment.

The main observations made by the Japanese workers with regard to the injurious effects of excess of vitamin A concentrates when given orally, were confirmed by a number of investigators, such as Harris and Moore (1928), Chevallier, Cornil and Chabré (1934), Simola and Kauppinen (1934), and Ypsilanti (1935).

In 1933, Collazo and Rodriguez, as well as Bomskov and Seeman (1933), described another remarkable lesion: The skeletons of rats which had received excess of vitamin A concentrates became so fragile that the animals incurred fractures of the long bones of the legs in the course of the limited movement possible in captivity in small cages. Davies and Moore (1934) found that sometimes the broken ends of the long bones were ankylosed with the formation of large irregular calluses. Further observations of these skeletal lesions were published by Strauss (1934), Hoff and Jeddeloh (1935), Vedder and Rosenberg (1938), and Weslaw, Wronski, Wroblewski and Wroblewski (1938).

More recently a further injury has been reported by Rodahl and Moore (1943) whose rats often died from severe internal hemorrhage

after receiving excess of rich sources of vitamin A. This bleeding was much more profuse and sudden than the diffuse bleeding in various membranes described by previous workers. Otherwise they found that the lesions produced varied remarkably according to the size of the rat and the magnitude and duration of the overdosage. Thus skin lesions, ranging from a slight roughening of the hair to soreness and alopecia were common at all ages. When the vitamin was given in the form of drops of concentrate into the mouth, peeling of the skin at the corners was frequently observed. Enteritis, emaciation and pneumonia was also observed. The more specific lesions, however, were found to be fracturing of the bones, seen most frequently in growing rats, — and hemorrhages, often seen in adult animals.

A number of workers report immediate loss of weight in rats given excess of vitamin A, and that soreness of the eyes and diarrhea may develop. These symptoms together with the frequent loss of use of the posterior extremities, have to some investigators resembled the condition of vitamin A deficiency.

Some authors find evidence of changes in the internal secretory glands in hypervitaminosis A. Thus Uotilla (1938) finds evidence of increased activity of the thyroid gland, Cornil, Chevallier, Paillas and Chouquet (1939) stressed increase in the basophilic cells of the anterior lobe of the hypophysis, and Cornil, Chevallier and Paillas (1939) report hypertrophy of the island tissue of the pancreas.

A temporary rise in cholesterol and in total lipids of the serum following large doses of vitamin A has been reported by several workers (Wendt (1936), Josephs (1944)), and Chevallier and Baert (1934) found that the basal metabolic rate was decreased.

Bomskov and Sievers (1933) observed no effect on the formation of blood in hypervitaminotic rabbits, while the sedimentation rate was decreased. The serum calcium and phosphorus has been reported not to be affected (Bomskov and Seeman (1933)).

Histologically Drigalski and Laubmann (1933) found degeneration of the renal tubules and glomeruli, of the testes, of cardiac muscle and to a small extent of hepatic cells in hypervitaminosis A. A number of investigators have also detected rarefaction of the bones with extreme thinning of the cortex (Bomskov and Seeman (1933), Wolback and Bessey (1942)), and increased deposits of fat in the reticulo-endothelial cells, especially the Kupffer cells of the liver and the spleen (Domagk and von Dobeneck (1933), Collazo and Rodriguez (1933), Drigalski and Laubmann (1933), Uotilla and Simola (1938), Noetzel (1939)). In the incisor teeth in hypervitaminotic rats, Irving (1949) has found reduced dentine formation and atrophy of the odontoblasts.

Simola and Kauppinen (1934) observed that large doses of vitamin A concentrates caused reduced weight gain also in guinea pigs.

It is generally agreed that recovery is prompt when the excessive intake of vitamin A is discontinued.

While it was well established that concentrated sources of vitamin A were toxic when given in great excess, it has been discussed whether vitamin A itself is responsible for the toxicity, and if so, whether it produces all or only some of the lesions which have been reported. Although it was not quite certain that vitamin A itself is poisonous, it was considered that the toxicity was at least closely associated with the vitamin in its concentrates. It is not at all unlikely, however, that crude fish liver oil may contain other substances than vitamin A which might produce ill effects upon experimental animals, regardless of the vitamin A content. Thus, Agduhr (1926, 1928), reported ill effects of cod liver oil on the muscles of various animals and stated that the oil was still injurious after its vitamin A had been destroyed.

As a result of investigations Yamamoto (1934), and later on Yosida (1937), concluded that it was the glyceride fraction and not the vitamin A which was responsible for the ill effect of many marine oils on rats. Hartwell (1927), who found that female rats suffered from uterine hemorrhages when allowed to become pregnant on diets rich in cod-liver oil, interpreted that this lesion was not caused by the vitamin A, but was a result of the destructive action of the oil on the vitamin E in the diet. Moore and Wang (1945) report that sperm oil is toxic to rats on account of the presence of cetyl alcohol. They also found that a stale specimen of sardine oil was toxic to rats although the oil was devoid of vitamin A.

Many of the concentrates which were used in the earlier experiments were not only rich in vitamin A, but had also an equally high vitamin D content, which is known to be toxic when given in great excess. It is, therefore, a question whether some of the lesions described are caused by the action of vitamin A, or vitamin D, or both of them, although the vitamin A source used by Rodahl and Moore was found to contain practically no vitamin D.

So far, therefore, the question was still unsolved whether vitamin A itself was responsible for the observed toxic effects, or whether there were some other components than the vitamin A present in the crude sources, which might be responsible for the toxicity. Experiments with comparatively pure concentrate, furthermore, have also suggested to some investigators that the vitamin may not be implicated after all. While Drigalski (1933) found that concentrates where the vitamin A had been destroyed by ultra-violet irradiation were harmless when given orally to rats, Matsuoka (1934) observed convulsions in rats after the injection of concentrates which had been freed from vitamin by oxidation or hydrogenation, and he found that a distillate containing the vitamin was non-toxic. Hamano (1935) prepared various crystalline derivates

of vitamin A, and found that toxic factors accompanied the vitamin A into the unsaponifiable fraction, but could be separated by their inferior solubility in methanol. In his experiment, the purified vitamin did not appear to be toxic. Finally, Vedder and Rosenberg (1938) found no close relationship between vitamin A content and toxicity, as judged by the incidence of bone fractures, when using distillates prepared from jew-fish liver oil. They believed that the fish liver oil concentrate contained an unidentified toxic substance which was responsible for the observed symptoms. On the other hand, Moore and Wang (1945), who tested the toxicity in young rats of pure vitamin A acetate dissolved in peanut oil, found that the toxicity increased by the increased doses of vitamin A, and that no ill effect was observed from the same oil when the vitamin A had been destroyed by aerating on a boiling water-bath.

Davies and Moore (1934) could not produce a toxic state in animals by giving huge amounts of carotene, although Sherwood and collaborators (1936) reported that excess of carotene stops oestrus and libido in rats.

As a result of comprehensive studies of the lesions in hypervitaminosis A in rats, using a crystalline vitamin A acetate, Moore and Wang (1945) found the most characteristic lesion in genuine hypervitaminosis A to be the skeletal fractures and hemorrhages. The fractures were found to occur most constantly in young growing rats, while no fractures were detected in their older animals. Hemorrhages, on the other hand, were frequent, although irregular in their incidence, distribution and severity in older rats. Bleeding was sometimes found in the limbs of rats which appeared to be in good health before they were killed. In others heavy internal hemorrhage into the viscera, or external hemorrhage through a minor wound, was rapidly fatal, as already described by Rodahl and Moore (1943). In several animals they observed rawness of the skin around the eyes, nose and mouth. They found that this injury was undoubtedly due to overdosing with vitamin A, but they considered this to be a less specific lesion than fracture or hemorrhage. In one of their experiments, exophtalmus was seen in all cases, while some investigators state especially that they have never seen this symptom in hypervitaminosis A. By X-ray examination they found that the bones had become abnormally thin in the hypervitaminotic rats. In one case there was detachment of the epiphysis from the shaft of the bone, otherwise they found that the fractures occurred most often near the centre of the long bones. They also examined the ash content of the pooled tibiae of the control group as compared with a group given excess of vitamin A, and found no significant difference, while Bomskov and Seeman (1933) report decreased mineral content of the bones in hypervitaminotic rats.

The uterine hemorrhage in pregnant rats given excess of cod liver oil, first reported by Hartwell (1927), was reproduced by Moore and Wang (1945) in animals given excess of cod liver oil, halibut liver oil,

or vitamin A acetate. They interpreted this hemorrhage as a special manifestation of the general liability to hemorrhage in hypervitaminosis A. In their experiments they found that even when the young were born alive, they were not reared unless the mother's ration was changed to a mixed diet of natural foodstuffs.

That the fractures can be caused by overdosing with pure vitamin A has been confirmed by Herbst, Pavcek and Elvehjem (1944), and Pavcek, Herbst and Elvehjem (1945).

In accordance with the findings of Rodahl and Moore (1943), and of Moore and Wang (1945), Walker, Eylenburg and Moore (1947) report the most characteristic lesion of hypervitaminosis A to be hemorrhages variable in intensity and distribution and also spontaneous skeletal fractures, which occurred mainly in young rats. Both these injuries were produced in various groups in the experiments, and the apparent effect of age reported by Rodahl and Moore (1943), and Moore and Wang (1945) was confirmed. Thus, at autopsy, the hemorrhages found in adolescent animals were more widespread and severe than those in young rats, but spontaneous skeletal fractures were absent. They also found that the older animals seemed to be in a poorer state of health than the young animals and the food consumption and hence their intake of vitamin A was smaller.

Another important observation was made by Light, Alscher and Frey (1944), who showed that hypervitaminosis A in rats was associated with a pronouced hypoprothrombinemia which could be corrected by giving vitamin K as 2-methyl-3-phytyl-1 : 4-napthoquinone. This observation has been confirmed by Walker, Eylenburg and Moore (1947), who concluded that a secondary deficiency of vitamin K may be induced by toxic excess of vitamin A. Any satisfactory explanation of the action of vitamin K in this case has not been given. They found that although rats given vitamin K invariably had normal prothrombin times, the action of vitamin K in preventing hemorrhage was only constant in rats which had initial body weights of about 85 grams and over. Among the smaller rats, several which were dosed with vitamin K had hemorrhages, and moreover, two of those not dosed with vitamin K which had hemorrhage, gave normal prothrombin times. When an additional supply of 50 mg ascorbic acid per rat per day was given, the mean prothrombin time was less than in the untreated group, but greater than in the group dosed with vitamin K.

Walker, Eylenburg and Moore (1947) further report that the blood in the hypervitaminotic rat appears to be thin and watery, the mean plasma/cell ratio for all animals given excess of vitamin A being 2.2 as compared with 1.2 for control animals. When vitamin K was given in addition to vitamin A the mean ratio was 1.6. It is interesting to note that the reported increase in the plasma/cell ratios found in the hyper-

vitaminotic rats was not only due to blood dilution subsequent to bleeding, as in young rats an increase in the plasma/cell ratios was not accompanied by hemorrhage. This observation that the hypervitaminosis A in rats is accompanied by an increased plasma/cell ratio is in agreement with the works of Poumeau-Delille (1943) who found a severe erythroblastic anemia in rats given toxic doses of vitamin A. In their experiment, there was a marked fall in both red and white blood cells, although platelet count was normal. Further, Poumeau-Delille states that there was no alteration in bleeding or coagulation times. It must be noted, however, that in the French laboratory the vitamin A was given by subcutaneous injection, whereas oral administration was employed in the case of Walker, Eylenburg and Moore.

While there is little in the literature suggesting any relationship between vitamins K and A in the normal animal, Thoenes (1935) has asserted that vitamin A and D are antagonistic, and Gross-Selbeck (1935) has reported that liberal dosing with vitamin A affords protection against injury through excess of vitamin D. Vedder and Rosenberg (1938) found that vitamin D (50,000 I. U. daily) gives protection against excess of vitamin A concentrate. Strauss (1934) on the other hand, found that the "decalcification" of the bones could not be prevented by vitamin D. Bomskov and Seeman (1933) report that excess of vitamin A in the form of "Vogan" resisted the rickets-curing capacity of vitamin D. Moore and Wang (1945) found that no benefit was derived from doses of 1 mg of calciferol daily, nor did they find any obvious effect on the development of hypervitaminosis A by varying the doses given of vitamin B complex, while other workers have found that an additional allowance of the vitamin B complex was beneficial to animals given excess of marine oil (Harris and Moore (1928), and Bell, Gregory and Drummond (1933)).

In 1922 Mouriquand and Michel reported noxious effect of large doses of cod liver oil in scurvy, claiming that cod liver oil antagonised vitamin C in guinea pigs, causing scurvy even when lemon juice was given. Collett and Eriksen (1938) supported this contention with regard to cod liver oil, but found that moderate excess of vitamins A and D had no injurious effect. Vedder and Rosenberg (1938) have pointed out that the symptoms which they observed in their animals given excess of fish liver oil, suggested the condition of scurvy, although normally the rat is not liable to scurvy since it is able to synthesize ascorbic acid. Thus growth failure and fragility of the bones are associated with hemorrhage both in hypervitaminosis A and scurvy. Furthermore, they found that ascorbic acid almost completely counteracted the toxic effect of jew-fish liver oil in rats.

Moore and Wang (1945) discussed the points of similarity and difference between the gross lesions in hypervitaminosis A and in scurvy, and they were unable to confirm the view that a secondary deficiency of

vitamin C can be detected in hypervitaminosis A by biochemical means. Thus they were unable with pure vitamin A acetate, either to repeat the observation of Vedder and Rosenberg (1938) that ascorbic acid affords protection against excess of vitamin A concentrate, or to detect any abnormalities in the ascorbic acid metabolism in hypervitaminotic rats. Walker, Eylenburg and Moore (1947) reexamined the effect of vitamin C on hypervitaminotic rats, and found that the ascorbic acid gave no protection against either hemorrhages or fractures.

With regard to the mechanism of the intoxication of vitamin A, very little is known. Moore and his collaborators conclude that it is due to too rapid absorption of vitamin A and not to direct toxic effect of large stores of vitamin A in the liver. In Walker, Eylenburg and Moore's experiments on rats, examination of the vitamin A content of the plasma failed to reveal any definite level of the vitamin A in the blood associated with distinct symptoms of hypervitaminosis A. Josephs (1944) concludes that experimental and clinical observations indicate the existance of a mechanism for the maintenance of a constant level of vitamin A in the blood, possibly related to the activity of the reticuloendothelial cells. He states that the occurrence of hypervitaminosis A in a child reported by him indicates either that some defect existed in the system responsible for the protective reaction, or else that intense prolonged overdosage is capable of injuring the regularity mechanism. He also suggests that it is not the total amount of vitamin A in the body that determines the presence of toxic symptoms, but the ingestion of amounts large enough to overwhelm the ability of the liver to remove it from the circulation.

2. Hypervitaminosis A in Man.

Little is known about the toxic effect of excess of vitamin A in man. Rodahl and Moore (1943), from chemical and biological investigations of the livers of polar bears (Ursus maritimus), and bearded seals (Erignathus barbatus) came to the conclusion that the illness which may promptly follow from eating these livers is due to intoxication from the huge amounts of vitamin A which they contain, since a consumption of about three-quarters of a pound would provide 7,500,000 I. U. vitamin A which, on analogy with rats, would be a toxic quantity. The symptoms of this acute polar bear liver poisoning, which may start within two to four hours, are drowsiness, sluggishness, irritability or an irresistible desire to sleep, severe headache and vomiting. In severe cases peeling of the skin around the mouth appears after twenty-four hours and may remain confined to the face, or the whole skin from head to foot may be involved.

In a more comprehensive study of the toxic effect of polar bear liver, Rodahl (1949, 1, 2,) confirming this conclusion, found that the livers

of Arctic mammals, which are known by the Eskimos to be poisonous, such as polar bear, bearded seal and Greenland fox, were very rich in vitamin A, while the livers of snow hare and walrus, which are considered non-poisonous by the Eskimos, contained only small amounts of vitamin A. In experiments on rats the toxic substance of bear liver was found to be identical with vitamin A, and ingestion of large quantities of bear liver led to the condition of hypervitaminosis A. The clinical symptoms of acute intoxication observed in rats following the ingestion of large single doses of vitamin A could well be compared with the symptoms observed in man following a single meal of bear or seal liver.

Rodahl and Moore (1943) also mentioned a man who took about 6,000,000 I. U. vitamin A daily for five days, in the form of halibut liver oil. He became severely ill, the main symptom being giddiness. The patient recovered within ten days after the ingestion of the halibut liver oil had been discontinued.

Some workers have reported that comparatively small doses of vitamin A may give rise to toxic symptoms in man. Thus Getz et al. (1939) reports that 2,000,000 I. U. vit. A in a single dose may give a dull headache, and Spiesman (1941) has seen adults who suffer from general malady with loss of weight and appetite on only 14,000 I. U. daily. On the other hand, others (Bicknell and Prescott (1947)) have given 144,000 I. U. daily for many weeks with no ill effects apart from an occasional transient diuresis at the beginning of the treatment. In children doses of 300,000 I. U. daily for several weeks or several months appeared to be harmless (Lehman and Rapaport (1940)).

Prolonged administration of vitamin A in large amounts may, on the other hand, be dangerous. Thus Josephs (1944) has reported a case of severe hypervitaminosis A in a three years old boy, who from the age of two or three months was given one teaspoon of halibut-liver oil daily, corresponding to approximately 240,000 U. S. P. units of vitamin A. It was found that his height was normal for his age, but his scalp hair was scarce, dry and coarse, and he had no hair on his body, nor had he any eyebrows. The condition was characterised by hepatomegaly, splenomegaly, hypoplastic anemia, leukopenia, increased serum vitamin A, increased serum lipids, advanced skeletal development and clubbing of the fingers. The erythrocytes sedimentation rate was 6 mm in one hour on one occasion, and 0 on two others. Roentgenograms of the chest and skull showed no abnormalities, but those of the limbs revealed considerable irregularity of the cortical structure. In the phalanges and metacarpals the cortex was extremely thin and the epiphyses of the upper end of the humeri and tibiae were mottled in appearance. It was found that when the extra supply of vitamin A was removed, the improvement was immediate. Within two months the appetite had improved, the patient had gained weight and his hair had begun to grow quite normally.

II. Experimental.

From the available literature it appears evident that there are still a great number of unsolved problems with regard to the condition of hypervitaminosis A, which need further investigation. In a great many instances the various workers have come to contradictory results, and a number of observations require closer examination. The conflicting reports suggest that variables in experimental procedures, at least to some extent, influence the manifestation of the toxic effect, and it has been questioned whether the reported signs of toxicity were in fact evidence of hypervitaminosis A or the result of an unknown toxic substance in the vitamin A preparations that were used. The great majority of investigators agree, however, that the condition of hypervitaminosis A is a reality. On the other hand, there appears to be considerable difference in opinion as to which symptoms and lesions may be considered specific for excess of vitamin A, and very little is known with regard to the mechanism of the toxicity.

1. Preliminary Investigations.

In a preliminary investigation (Rodahl 1949, 1, 2) the purpose of which was to study the toxic effect of polar bear liver, which was found to be due to its high content of vitamin A, the following observations were made with regard to the manifestation of hypervitaminosis A.

In rats, some of the clinical symptoms constantly occurred in direct connection with the first large doses of vitamin A, as some sort of acute intoxication, while other symptoms occurred when the excess of vitamin A had been given to the animals in repeated doses for several days. The symptoms could thus be divided into symptoms of "acute intoxication" and symptoms of more "chronic intoxication". Of these, the "acute intoxication" symptoms appeared to be signs of general malady, and unwell-being with changes in the pelts, drowsiness, weakness and decreased activity. These symptoms may well be compared with those observed in man and dogs following a single meal of bear or seal liver.

The symptoms of "chronic intoxication" were found to be: reduced weight gain, limping, stiffness in the limbs, alopecia, soreness and bleeding in the skin, and eye symptoms such as exophtalmus, loss of hair and soreness around the eyes, as well as swelling of the palpebrae, and finally fractures. All these symptoms appeared to be of a more serious nature, indicating pronounced pathological changes in the organs. Of the symptoms following continued ingestion of excess of vitamin A, the stiffness in the limbs occurred first followed by eye symptoms, limping, alopecia and finally fractures.

Absence of the normal weight gain in young growing rats was found to be one of the first symptoms following ingestion of large

doses of vitamin A. During a period of thirty days it was found that the higher the excess of vitamin A, the greater was the reduction in the weight increase. 362 I. U. vit. A/g body weight thus reduced the weight increase to approximately half of the normal, and 476 I. U./g to approximately one third of the normal. This reduction of the weight increase was probably due to reduced food consumption as a result of loss of appetite in the experimental animals. A slight hypochromic anemia was found in some of the hypervitaminotic rats, but in most of these cases hemorrhages were found which could explain the anemia. In practically all cases the blood coagulated normally, as judged by the rough clinical method. Additional supply of vitamin K had no effect on the hemorrhages produced by bear liver oil. The prothrombin time was examined in one of the rats receiving bear liver oil, and was found to be normal. The sedimentation reaction was found to be less in the rats receiving excess of vitamin A as compared with normal rats.

No pathological changes were found in the differential blood counts as compared with normal rats. In many cases macroscopical postmortem examination revealed surprisingly few pathological findings in view of the pronounced clinical symptoms which had manifested themselves in the rats receiving excess of vitamin A. Otherwise the most significant symptoms were hyperemia, hemorrhages and fractures.

The hemorrhages were general, both subcutaneous and visceral. Most of these hemorrhages were scattered and relatively small, and in none of the cases they appeared to be fatal.

Fractures occurred frequently in rats receiving excess of vitamin A. This symptom occurred late in the experiment, usually twenty to forty days from the beginning of the experiment after a total gross consumption of 500,000 to 1,000,000 I .U. vitamin A, after signs of pathological changes in the bones had manifested themselves for some time, in the form of stiffness in the legs and limping. The fractures occurred in most of the cases at the proximal end of the tibia and the fibula. Complete healing of the fractures was found to take place without any hindrance, just as quickly as in normal rats, even when the vitamin A was given continuously in unchanged doses.

The ash content of the bones calculated on a dry basis was approximately the same in rats given excess of vitamin A, as compared with the control rats, which again was practically identical with that of the rats receiving bear liver oil where the vitamin A had been destroyed, or bear liver where the vitamin A had been removed. Furthermore, the calcium and phosphorus content of the ashed bones was practically the same in all these cases.

Of the microscopical findings, hyperemia, scattered hemorrhages in the internal organs, slight degeneration of the renal tubules, and sudanophil deposits in, and in particular between, the liver cells, appeared to

be the most constant findings. In some cases free blood was seen in the renal tubules, thus explaining the hematuria which was frequently observed in the hypervitaminotic rats.

Systematical X-ray examination of the long bones in the hypervitaminotic rats at the various stages of the experiment, showed that the bones gradually became abnormally thin. This reduction in the diameter of the bones particularly involved the bone shaft, while the epiphysis appeared to be more or less of normal width. This was particularly noticeable at the proximal end of the tibia and the fibula. This decrease in the diameter, which occurred early in the hypervitaminotic rats, involved the entire bones including the cortical shadow, which was often only represented by a narrow white line. In some cases it was observed that the cortical shadow was absent at both ends of the bones, and when the periost was removed at postmortem, only spongy and no compact bone was found at the mentioned places.

Apart from the described reduction in the diameter of the bone shaft, a deformity was simultaneously observed, taking the form of a depression in the compact bone usually 8—10 mm from the proximal end of the tibia. At this particular place fracture usually occurred at a later stage. Other typical locations of the fractures were: at the middle of the humerus, and at the distal end of the radius and the ulna.

As judged by the roentgenograms, it appeared as if there was no lack of calcium in the bones. In fact there appeared to be abnormal richness of calcium in some cases in the metaphyses.

No ill effect was observed in the rats given fat-free bear liver deprived of its vitamin A content, or in rats given bear liver oil where the vitamin A had been destroyed.

2. Problems, and Plan for the Investigation.

A majority of the previous workers have mainly used rats or mice for their experiments on hypervitaminosis A, and comparatively little information is available as to the manifestation of hypervitaminosis A in other experimental animals. An endeavour to elucidate the problems connected with this condition seems, therefore, desirable in experiments on different types of experimental animals particularly since it has been suggested by some investigators that different species vary greatly in degree of sensativity to, and also possibly in the type of changes produced by, excessive amounts of vitamin A (Josephs, 1944).

The purpose of the present investigation, therefore, was to make a further study of the nature of the condition of hypervitaminosis A, including a systematical study of the symptomatology of hypervitaminosis A, the toxic doses and the mechanism of the toxic effect in rats, mice, guinea pigs, rabbits, and chicken.

The problems exposed themselves as follows:

Firstly: is vitamin A itself toxic, or are there possibly other toxic substances present in liver oils, which might be responsible for the observed toxic effect.

Secondly it would be of interest to determine the approximate dose of vitamin A producing toxic symptoms in animals at different stages of development, as it has been previously suggested that young and adult animals may react differently on overdosage with vitamin A.

It would further be of interest to study the effect of large single doses of vitamin A given in the course of a short period of time compared with the effect of prolonged administration of excess of vitamin A, and to examine whether single massive doses of vitamin A may have an immediate lethal effect as the result of acute intoxication, and whether single massive doses might give rise to permanent pathological changes in the organism.

Since it has been suggested that different experimental procedures may influence the clinical manifestation of hypervitaminosis A, the importance of different modes of application of the vitamin A for the production of the symptoms should also be subject to investigation, including the effect of subcutaneous injection of vitamin A compared with oral administration.

The nature of the clinical manifestation and the pathological changes in the organism seems to be a problem of major importance and should be subject to detailed studies.

The next problem would be to classify and group the more constant clinical symptoms of hypervitaminosis A and to ascertain whether all of these symptoms are directly caused by the vitamin A.

It would also be desirable to examine in further detail the relation between hypervitaminosis A and the action of other vitamins on account of the many conflicting reports on this point.

In an endeavour to solve these problems, and with the results of previous experiments in view, it was decided to proceed with the investigations as outlined in the following:

Since most of the present knowledge with regard to hypervitaminosis A had been gained from experiments on rats, it was considered practical to confine the main studies to these animals.

As a general source of vitamin A it was considered practical to use purified whale liver oil concentrate since it had been found in previous experiments (Rodahl, 1949, 2) that this source of vitamin A was readily consumed by the animals, and contained no vitamin D. The possibility of introducing the condition of hypervitaminosis D was thus ruled out in the experiments where this source of vitamin A was used. The concentrate contained approximately 200,000 I. U. vit. A/g, and the vitamin A

content was found to remain quite constant when stored in refrigerator temperature.

It was considered natural first of all to examine the effect of this whale liver oil concentrate compared with the effect of vitamin A in its purest available form.

In order to decide whether vitamin A itself was responsible for the toxic effect, the various fractions of the whale liver oil concentrate were to be tested on rats, including whale liver oil concentrate where the vitamin A had been destroyed.

For the purpose of studying the importance of the different modes of application of vitamin A for the production of the symptoms, the effect of the different methods for oral administration of vitamin A, such as given by dropping pipet, catheter, or mixed in the basal diet, were to be examined and compared with the effect of parenteral administration of vitamin A in different groups of rats.

In order to determine the approximate toxic dose of vitamin A, and the effect of excess of vitamin A in young and adult rats, the effect of varying doses of vitamin A were to be studied under otherwise identical conditions in different groups of rats, at different stages of development. As far as possible these experiments were to be carried out simultaneously so as to make the results strictly comparable when the doses were expressed in terms of I. U. vit. A per gram body weight.

Simultaneously examinations were to be made of the general condition of the animal, the growth, appetite and food consumption, the skin and its derivates, the eyes and their surroundings, the teeth and the skeletal system, the blood and circulatory organs, the parenchymateous organs, the internal secretory glands, the intestinal tract, the urogenital organs, and the fertility and the development of the young.

To enable the study of the progressive changes caused by excess of vitamin A, the rats were to be killed and examined at various stages of the experiment.

For the purpose of studying the fertility in hypervitaminotic rats and the young from hypervitaminotic mothers, groups of adult rats given different doses of vitamin A were to be mated, and the young would be killed and examined at various stages of development.

Daily clinical examination of the animals throughout the experiments would enable a systematical study of the clinical manifestation of hypervitaminosis A in relation to the vitamin A consumption. By altering the doses of vitamin A and by varying the conditions of the experiments as well as the sources of vitamin A, it was considered possible to obtain an impression of which symptoms depended directly on the vitamin A, and which symptoms might be indirect rather than direct results of the overdosage.

Careful postmortem examination was to be carried out followed by histological examination of the internal organs for the purpose of studying the changes in these organs in relation to the severity of the clinical symptoms, the magnitude and degree of the overdosage.

The study of the relation between hypervitaminosis A and the action of other vitamins should in particular include a systematical comparison between the symptoms of hypervitaminosis A and those of hypovitaminosis C, and an examination of the effect of additional supply of this vitamin in animals given excess of vitamin A. For the further study of the similarities between hypervitaminosis A and scurvy, a special series of experiments were to be carried out on guinea pigs given excess of vitamin A, scorbutic diet, a combination of scorbutic diet and excess of vitamin A, — and finally additional supply of vitamin C in addition to excess of vitamin A.

For the purpose of comparison, similar studies as outlined for the rats were also to be carried out on mice, guinea pigs, rabbits, and birds.

On the basis of the information compiled from all these experiments the pathogenesis of the toxicity would be subject to discussion, considering that a combined study of the functional and morphological findings would offer the best possible chance to elucidate further this complicated problem.

3. Material, Methods and Technique.

The investigations described in the present publication involve a total number of 253 rats, of which 37 were normal controls. An additional material consisted of 20 mice, 38 guinea pigs, 6 rabbits, and a group of 6 cockerels. Of these 2 mice, 8 guinea pigs, 2 rabbits, and 3 cockerels were used for control purposes.

Postmortem examination was carried out in 210 of the rats, 8 of the mice, all of the guinea pigs, 4 of the rabbits, as well as all of the cockerels.

88 rats, 3 mice, 26 guinea pigs, 4 rabbits, and 4 cockerels were subject to histological examination.

Further details regarding the material are given in the respective chapters.

The arrangement and the conditions of the experiments will be described in detail in connection with each experiment. The experimental animals, as well as the diet and dosage will also be considered in the respective chapters. In this chapter only the general methods will be dealt with.

a) **Clinical Examination:** Each experimental animal was carefully examined daily, including observations of the general condition, the condition of the pelt, the eyes and their surroundings, as well as examination of any abnormalities such as limping, fractures, hemorrhages, paralysis, diarrhea, soreness of the skin, and

alopecia. The animals were usually weighed every other day, and roentgenograms were taken of the long bones at intervals.

b) **Postmortem Examination:** In all cases where the animals died, the organs were examined as soon after death as possible. Otherwise in the case of rats and guinea pigs, they were anesthetized by ether for a few minutes to allow blood samples to be collected from living animals for examination. Immediately afterwards they were killed by decapitation. The internal organs were quickly removed and placed in 4 % formalin. This short-lasting ether anesthesia was found to cause no degeneration of the internal organs in rats which for control purposes were compared with rats killed without ether anesthesia.

Microscopical slides were prepared from the preserved organs, such as the liver, kidney, adrenals, and other internal secretory glands, intestines, spleen, lungs, heart, as well as the bones and teeth of both experimental and normal control animals for histological examination.

The following staining methods (B. Romeis, 1924; F. B. Mallory, 1938) were used: hematoxylin-eosin, van Gieson, Mallory's staining, Kossa's staining, Best's glycogen staining and Turnbull's staining for blood pigment. Frozen sections of some of the internal organs, such as the liver, adrenals and in some cases the kidney, spleen, pancreas, intestines, lungs, heart and the thymus gland were stained by sudan III. In the present investigation, mitochondria staining was not used, and the applied staining methods allowed only marked degenerative changes to be recognised. In the case of bones and teeth, the vacuum method described by J. Wærhaug (1949) was applied for decalcification using formic acid (R. D. Lillie, 1948).

In the cases when the weight of the adrenals was recorded, they were carefully removed immediately after death, and quickly placed in a small glass tube supplied with a cork, and immediately weighed. In all cases the left adrenal was weighed, microscopical slides being prepared from the other adrenal. The weight of the adrenal was expressed in per cent of the body weight of the animal.

For the determination of the vitamin A content of the internal organs, the organs were removed immediately after death, weighed, and extracted by acetone in a Soxhlet's apparatus in a dark room, and the vitamin A was determined spectrographically in the fat. For the determination of vitamin C the phenol-indophenol method was applied, using 10 % trichloracetic acid for extraction.

c) **Blood Examination:** For the routine examination of the blood in rats and guinea pigs, samples were collected from the animal under ether anesthesia just prior to being killed, as already described. In a few cases, blood samples were collected from the rats tails. In the case of rabbits, blood was taken from the marginal veins of the ear.

Hemoglobin was determined in all cases by the same Zeiss Ikon hemoglobinometer (standard: 13.8 g hb/100 ml blood = 100 %). Blood counts were made in the usual manner (red blood cells) and the colour index calculated according to the formula:

$$\frac{\text{Hb \%}}{\text{mill. red blood cells per } 1/1000 \text{ ml} \times 20}$$

In a number of cases blood smears were taken for differential counts. For a rough determination of the coagulation time, drops of blood were placed on a glass and the time was noted when the coagulation was complete. In some cases a long Pasteur pipet was used for this purpose. The prothrombin time was determined by Quick's method (1935) using thromboplastin, produced by the technique described by Owren (1949). For a rough determination of the serum colour the Meulengracht's test was used (H. C. Gram, P. Iversen, and E. Meulengracht (1937)).

For the determination of ascorbic acid, vitamin A, total base, calcium, phosphorus, and iron in the serum from rats and guinea pigs, blood from the femoral artery was collected in the following manner: under light ether anesthesia the skin was carefully removed at the groin, the femoral artery was opened and as much blood as possible was collected directly into centrifuge glasses which had been thoroughly rinsed in glass distilled water. The blood was centrifuged immediately after coagulation, and the serum was transferred into small test tubes.

For the determination of the ascorbic acid in the serum, the method described by J. Bøe (1946) was applied. The ascorbic acid content was determined in fasting animals as well as in non-fasting animals.

Vitamin A was determined in the serum by a modification of the usual antimony trichloride method (modified by E. Kvalheim, personal communication): The serum was weighed and an equal amount of absolute alcohol was added. The alcohol-serum mixture was then transferred to a separation funnel and the test tube was rinsed with 10 ml petroleum ether, which was added in the alcohol serum mixture. It was shaken vigorously for several minutes, and extracted thoroughly three times with petroleum ether. All the extracts were collected and washed twice by water and transferred to a 100 ml flask. The alcohol and petroleum ether were then evaporated off on a water bath in vacuum. The fat was dissolved in 2 ml petroleum ether which was also evaporated off on a water bath in vacuum. The fat was allowed to cool and immediately the air was let into the flask it was dissolved in 1 ml petroleum ether. 0.2 ml of this solution was transferred for analysis into the container. Two drops of acetic acid anhydrate were added and the 2 ml antimony trichloride. The time was taken by a stop watch, and the reading was made in a Zeiss Pulfrich photometer. Reading was done after fifteen, thirty, sixty and ninety seconds, and the calculations were carried out according to the table and the standard curves.

For the determination of the total base in the serum, the electrodialysis method (Keys (1936)) was used. Calcium was determined in the serum by the method of Kramer—Tisdall (1921), and phosphorus by Kuttner and Lichtenstein's method (1930). Bood sugar was determined by Hagedorn-Jensen's method (1923), and the serum iron by Heilmeyer and Plötner's method (1937), using o-phenanthroline hydrochloride as indicator. In a few cases the blood urea was determined in the serum by the urease macro-method (Van Slyke and Cullen (1916)).

d) **Determination of Ash, and Mineral Contents of the Bones:** The femur was removed immediately after death, cleaned and weighed. The bones were dried until a constant weight was reached at a temperature of 104° C. After this the bone was ashed at 800° C until constant weight.

The ash was dissolved in 1—n. HCl in the porcelain vessels. The solution was transferred into measure flasks and made up to a certain volume with distilled water (usually 50 ml). A certain amount was taken out, in which the calcium was determined by Kramer-Tisdall's method. The phosphorus was determined by Kuttner and Lichtenstein's method.

e) **Urine Examination:** At intervals urine samples were collected during a period of 24 hours from the various groups of rats by placing the animals in specially constructed cages, where the urine was collected without being contaminated by feces. In some cases urine samples were also collected by careful puncture of the urinary bladder in ether anesthesia. The urine was examined with regard to the presence of protein, blood and sugar. In some cases the Schlesinger's test was made.

Urine samples were also collected in the same manner for the determination of the excretion of ascorbic acid during twenty-four hours, in flasks containing

metaphosphoric acid. The ascorbic acid content was determined by the phenol-indophenol method.

f) **Feces Examination:** At intervals feces were examined by the benzidine test as follows: Fresh benzidine reagent was prepared in the usual manner. A sample of feces was smeared out on a glass slide, one drop of the benzidine reagent was added, and the time noted by stop watch.

In some cases feces was collected during 24 hours periods, weighed and extracted by acetone in a Soxhlet's apparatus in a dark room, and the vitamin A content was determined in the fat spectrographically and by the antimony trichloride method.

4. Results.

A. Hypervitaminosis A in Rats.

Experimental Animals.

For the following experiments both albino and piebald rats of both sexes and at different stages of development were used. The rats were taken from the main stock of animals that had been living their entire life on an adequate basal diet.

All rats received daily as much as they wanted of the same ordinary adequate basal diet as had been used for the stock animals. The food had the following composition:

Oatmeal	2200 g
Corn meal	4000 »
Coarse wheat flour	3000 »
Wheat embryo	2000 »
Coarse rye flour	3000 »
Dried milk (skimmed)	4800 »
Sodium chloride	102 »
Calcium carbonate	102 »
Dried yeast	800 »
Peanut oil	500 »

In addition to this all rats in these experiments received two drops of a cod liver oil mixture once a week, corresponding to 150 I. U. of vitamin A, and 100 I. U. of vitamin D per rat, regardless of other sources of vitamin A. Whole maize was given once or twice a week, and ordinary bread once a week. Fresh milk and water was given every day and occasionally cabbage and carrots. Liver of pig, ox or calf was given once a week.

The rats were kept separated in special cages for each group, and all experimental animals lived under identical conditions. A number of experiments were carried out simultaneously, which allowed strict comparison. In most experiments the rats were weighed every other day, and in all cases the rats were examined clinically every day.

The vitamin A oil was given to the rats mixed in the basal diet, or by dropping pipets, or by specially constructed catheters, which were accurately adjusted, so as to allow exact dosage. In some experiments the oil was given both mixed in the basal diet and by pipet or catheter. In some cases it was necessary to give a relatively large mount of oil and the oil was then given to the rats by two to three drops at a time at intervals.

1. *The Effect of Liver Oil Concentrates and Highly Purified Vitamin A Preparations.*

a) The Effect of Liver Oil Concentrate.

Experiment 1.

In a previous investigation (Rodahl, 1949, 2), whale liver oil concentrate given to young rats in amounts corresponding to 18,000—31,000 I. U. vit. A daily, or 350—470 I. U./g body weight, produced symptoms and postmortem findings as described on page 15. In this experiment the effect of whale liver oil concentrate was reexamined in rats.

A group of four young rats with initial body weights between 60 and 65 grams were given whale liver oil, in addition to the adequate basal diet, in amounts corresponding to 50,000 I. U. vitamin A daily, i. e. 6 drops of the oil mixed in the diet, and 4 drops given by dropping pipet daily. This dose of vitamin A corresponded to between 833 and 500 I. U./g body weight daily.

For control purposes, two rats of approximately the same age as the experimental animals (initial body weight 64.0 and 63.0 grams) were used. They were given the ordinary adequate basal diet, and vitamin A and D in optimal growth doses. The control rats remained normal and free of any symptoms throughout the experiment, which lasted 24 days. The average daily weight increase was 3.40 grams (3.33 and 3.46). By postmortem examination, normal organs were found. By microscopical examination no pathological findings were detected. By sudan III staining of the adrenal the normal deposits of sudanophil droplets were seen in the cortex. The hemoglobin was 102 %, and the ascorbic acid content of the serum was 0.54 mg per 100 ml. The ash content of the femur was 53.5 % calculated on a dry basis, and the calcium and phosphorus contents of the ash were 36.3 % and 17.4 % respectively.

In all rats in the group given whale liver oil concentrate, the mentioned doses of vitamin A proved lethal after 26 to 30 days. In all cases there was a marked reduction in the weight gain, and the symptoms observed as well as the postmortem findings were similar to those reported from the previously mentioned experiment with whale liver oil (Rodahl, 1949, 2).

On the 24th day of the experiment, urine was collected with the aid of a specially constructed metabolism cage from all rats in this group during a 24 hour period. The total amount of urine was 12 ml, corresponding to 3 ml/rat. The Heller's test was distinctly positive, and the Esbach test showed 0.5 ‰.

The clinical symptoms and postmortem findings in the individual rats in this group were as follows:

In one of the rats soreness was observed around the eyes and mouth, and fractures of the right hind leg, as well as the left fore leg were clinically diagnosed at the end of 19 days. The average daily weight increase was approximately 1 gram per day.

When moribund on the 27th day of the experiment, the rat was killed and examined in the usual manner. By postmortem examination, fractures of the left

fore leg, as well as both hind legs were detected, with large hemorrhages around the fractures. The liver appeared fatty on the cut surface. Otherwise no pathological findings were made by macroscopical examination.

Microscopical examination revealed the following findings:

Liver (stained by sudan III): Dense deposits of large sudanophil droplets were seen, mostly between the liver cells (in the Kupffer cells), and only in a few places the droplets were seen in the actual liver cells (see ill. 28, where similar findings in an other hypervitaminotic rat are illustrated)

Adrenal (stained by sudan III): Sudanophil droplets were quite evenly distributed throughout the adrenal cortex. Slides stained by hematoxylin-eosin revealed a large number of vacuoles of varying size throughout the adrenal cortex, as well as considerable hyperemia in the adrenal (see also ill. 39—43).

Kidney: Moderate degree of hyperemia, and a few scattered red blood cells in the space of Bowman's capsule in some places (see also ill. 32).

Testis: No definate signs of degeneration of the testis could be detected. In the epididymis a large number of sperms were seen.

Bones (stained by hematoxylin-eosin): The slide was taken from the fractured tibia. There was marked hyperemia and great irregularity in the bone structure, and large subperiostal hemorrhages in the tibia, as well as in the fibula. There was normal density of bone cells. In the metaphysis there appeared to be normal richness in calcium, the spicules were extremely thin and there was a large number of osteoclasts. There was marked hyperemia with dilated blood vessels, and complete absence of compact bone near the metaphysis. The compact bone in the bone shaft was chaotically arranged, with large Howship's lacunae. The cortex was fractured in several places and old hemorrhages were seen, as well as large callus formation (ill. 46 shows similar bone changes in another hypervitaminotic rat).

The hemoglobin was 88 %, and the ascorbic acid content of the serum was 0.00 mg per 100 ml.

In the second rat, limping and marked weakness was observed on the 17th day, and distinct fractures were detected at the end of 19 days. The rat died at the end of 26 days, at which time the weight was approximately 7 grams less than at the beginning of the experiment, i. e. an average daily loss of weight of 0.27 grams. By postmortem examination, marked alopecia was observed over large areas of the abdomen. There were fractures of both tibiae, with large hemorrhages around the fractures. A few hemorrhages were seen in the intestinal wall. The adrenals were enlarged, and accessory adrenals were seen. The lungs were fiery red.

In the third rat, limping was observed on the 15th day, preceded by an increasing weakness. The average daily weight increase was 1.3 grams. When moribund it was killed on the 30th day of the experiment after having been fasted 24 hours. By postmortem examination, no significant pathological findings were revealed. The adrenals were enlarged and accessory adrenals were found. The hemoglobin was 91 %, and the fasting blood sugar was 156 mg per 100 ml. The serum iron was 100 γ %.

In the fourth rat, limping was observed on the 17th day, and two days later there were distinct fractures of all four extremities. It died at the end of 26 days, at which time the weight was approximately 5 grams less than at the commencement of the experiment, i. e. an average daily loss of weight of 0.2 grams

By postmortem examination, soreness was observed in and around the nose, as well as marked alopecia around the anus and over the abdomen. There were large hemorrhages around the fractures. The lungs were fiery red.

The ash and mineral contents of the femurs for the different rats in this group are given in table 2.

b) The Effect of Highly Purified Vitamin A Preparations.

Experiment 2.

For the purpose of comparing the effect of whale liver oil concentrate with the effect of vitamin A in its purest available form when given in equivalent amounts with regard to the vitamin A content, a group of three rats of the same initial body weight as used in the experiment with whale liver oil concentrate (approximately 60 grams) were given vitamin A acetate obtained from saponified and purified maritime oil concentrate dissolved in a minimum of peanut oil, yielding 300,000 I. U. vit. A/g, in amounts corresponding to approximately 52,500 I. U. vitamin A daily. In order to maintain identical experimental conditions, the oil was given to the rats in the same manner as the whale liver oil concentrate, i. e. both mixed in the basal diet and by dropping pipet.

One of the rats in this group became moribund at the end of 21 days. During the entire experiment, the average daily weight increase was 0.7 grams. On the 9th day it appeared very weak, looked scruffy, limped, and the eyes were sore and practically closed by swelling of the palpebrae. Alopecia was observed at the end of 19 days, at which time fractures were also diagnosed.

When moribund, the rat was killed and examined. There was oedema around the eyes, and fractures in all four extremities. Otherwise no significant pathological findings were revealed by macroscopical postmortem examination.

The blood coagulated within 5 minutes. The hemoglobin was 84 %, and the ascorbic acid content of the serum was 0.39 mg per 100 ml.

The second rat in this group died at the end of 11 days, at which time the weight was approximately 8 grams less than at the commencement of the experiment, i. e. an average daily loss of weight of 0.7 grams. On the 4th day the rat limped, and the following day exophtalmus was observed. On the 6th day there were fractures of both hind legs. On the 9th day, the general condition of the animal was particularly poor, it was weak and scruffy, both eyes were sore, and practically closed by swelling of the palpebrae.

By postmortem examination, alopecia and soreness were observed around the mouth. There were fractures of all four extremities, with large hemorrhages around the fractures. There were subcutaneous hemorrhages over the abdomen and thighs. There was general muscular dystrophy, the bones were brittle and broke easily when grasped with an ordinary forceps. The lungs were fiery red.

By microscopical examination the following findings were made:

Liver (stained by sudan III): Massive deposits of sudanophil droplets both in and between the liver cells, particularly in the Kupffer cells.

Adrenal (stained by sudan III): Deposits of sudanophil droplets in a narrow strip in the zona glomerulosa of the adrenal cortex.

Kidney: Marked hyperemia; the cells of the convoluted tubules showed swelling and granularity. A few red blood cells in the spaces of Bowman's capsules.

Testis: No pathological findings.
Tibia: Marked hyperemia, and signs of increased osteoclastic activity.
Costochondral junction: Clubbing, hyperemia and old hemorrhages. Irregularity of bone structure.
Teeth: Marked hyperemia in pulp and hemorrhage in several places, degeneration and necrosis of odontoblasts, and disunion of odontoblasts and dentine (see ill. 52).

The third rat in this group was killed when moribund on the 11th day, having lost 8 grams in weight from the beginning of the experiment, corresponding to an average of 0.7 grams daily. Distinct limping was observed on the 4th day, and the following day eye symptoms occurred. On the 6th day fractures of both hind legs were diagnosed. The general condition became rapidly worse. There was marked weakness and the rat looked scruffy, both eyes were practically closed by swelling of the palpebrae. When moribund it was killed and examined in the usual manner. There was marked alopecia and soreness around the mouth and fractures of all four extremities with large hemorrhages. Otherwise no hemorrhages were seen. The lungs were fiery red, but no signs of pneumonia were detected. The liver appeared fatty on the cut surface. Otherwise no pathological findings were revealed in the internal organs by macroscopical examination.

By microscopical examination the following pathological findings were made:

Liver (stained by sudan III): Comparatively slight deposits of sudanophil droplets, mostly between the liver cells.
Adrenal (stained by sudan III): Very slight deposits of sudanophil droplets in the cortex.
Kidney: Very marked hyperemia. The same degenerative changes as described for the previous rat of some of the renal tubules, were seen, some of which were filled with amorphous masses and a calcium-like deposit. Erythrocytes in the spaces of Bowman's capsules in some places, as well as in some of the renal tubules (see ill. 32, 33).
Skin from Head: Marked hyperemia, and massive hemorrhages around the root sheaths of the hair (see ill. 38).

The hemoglobin was 96 %, and the blood coagulated normally. The total base in the serum was 149.5 milliequivalents/liter.

The ash and mineral contents of the femurs of the rats in this group are given in table 2.

Experiment 3.

The purpose with this experiment was to examine the effect of highly concentrated and purified sources of vitamin A, produced from shark-liver oil, dissolved in a minimum of peanut oil, yielding 640,000 I. U. vit. A/g. The oil contained practically no vitamin D.

Four drops of this oil, corresponding to 60,000 I. U. vit. A, was given daily to a group of 4 rats with initial body weights between 79.5 and 91 grams (average 85.5 grams) by dropping pipet, in addition to the usual adequate basal diet. Another group of two rats with similar initial body weights was used as control, and was given 4 drops of peanut oil by dropping pipet in addition to the usual basal diet, without excess of vitamin A.

In the first mentioned group given excess of vitamin A, one rat was killed and examined at the end of 28, one at the end of 38, and two at the end of 42 days. The average daily dose of vitamin A was 697 I. U. vit. A/g body weight. The average weight remained practically unchanged throughout the experiment. The secreted amount of ascorbic acid in the urine collected from the 5th day was 0.0 mg per rat in 24 hours.

By clinical examination, similar findings were detected as for the rats in the previously described experiments 1 and 2.

In the individual rats in this group the following clinical symptoms were observed:

Rat No. 1: Swelling and soreness of the left eye was observed the second day, — the eye being practically closed. Two days later both eyes were affected. An increasing weakness and scruffiness was observed from the 6th day. On the 9th day marked soreness around the nose (sore crusts) as well as alopecia around the mouth and neck, and a limping gait was observed. On the 15th day there was also marked loss of hair around the anus, and fracture of the right fore leg. Three days later the rat limped on all four extremities, at which time the loss of hair was marked and general. At the end of 33 days, fracture of the left hind leg was also diagnosed, at which time the hair had started to grow out again, and the soreness had improved. This improvement occurred in all rats in this group towards the conclusion of the experiment.

Rat No. 2: The rat looked scruffy on the 6th day of the experiment, and there was soreness around the mouth. Alopecia around the nose and on the neck, as well as a limping gait was observed on the 9th day. An increasing weakness was observed from the 12th day, at which time both eyes were practically closed from swelling of the palpebrae. Loss of hair around the anus was observed on the 23rd day. No fractures were observed in this rat.

Rat No. 3: Soreness around the nose and eyes, loss of hair around the mouth and on the neck, as well as limping was observed on the 9th day. Three days later it looked very scruffy and weak, both eyes being practically closed from swelling of the palpebrae. Loss of hair around the anus was observed on the 15th day. The general condition became gradually worse, although it again improved towards the conclusion of the experiment, at which time the soreness and alopecia, like all other rats in this group, was less marked. Fractures of the left fore leg were observed in this rat.

Rat No. 4: Eye symptoms were observed on the third day, and limping on the 6th day, at which time the rat was weak and scruffy. On the 9th day marked soreness and alopecia was observed, as described for the other rats in this group, at which time a fracture of the right clavicula was observed by X-ray examination. The general condition gradually became worse, with increasing soreness and loss of hair and eye symptoms. At the end of 15 days there was complete loss of hair around the mouth, and around the anus as well as on the medial side of the fore legs. At the end of 19 days there was fracture of the left hind leg, and at the end of 27 days of both hind legs. Towards the conclusion of the experiment, when the general condition appeared somewhat improved, there were fractures of all four extremities.

By postmortem examination emaciation and fatty appearance of the cut surface of the liver was found in all cases. Abscesses in the kidney were seen in three of the rats, and accessory adrenals in one. In one case the lungs were fiery red, and in this case swollen visceral lymph glands were also seen. In one rat the pancreas appeared enlarged, the costochondral junction was clubbed, and slight

hemorrhages were seen in and around one of the knee joints. Otherwise, no hemorrhages were observed.

Histologically similar findings were detected in the two rats examined, as described in the hypervitaminotic rats in experiments 1 and 2:

Liver (stained by sudan III): Dense deposits of sudanophil droplets were seen throughout the organ. Some of the droplets were very large, and were practically all situated between the liver cells, particularly in the Kupffer cells.

Adrenal (stained by sudan III): A band, distinctly defined, of sudanophil droplets, brightly stained, was seen in the zona glomerulosa of the cortex, otherwise there were scattered sudanophil droplets throughout the cortex of moderate density. When stained by hematoxylin-eosin, a large number of vacuoles were seen throughout the cortex.

Salivary glands and Lymph glands: normal findings.

Pancreas: Hyperemia.

Kidney: Moderate hyperemia.

Tibia: Marked hyperemia, periostal hemorrhages with deposits of pigment. In one place in the metaphysis, bone tissue was substituted by a loose connective tissue in which hemorrhage was seen in one place. Complete irregularity of bone structure; signs of increased osteoclastic activity with enlarged Howship's lacunae.

Ribs: Swelling of costochondral junction, with great irregularity of bone structure.

Teeth: Marked hyperemia in pulp where also blood pigment was seen. The odontoblasts were quite irregularly arranged, and red blood cells were seen scattered in between them.

In the control group, the rats remained normal and free of any symptoms throughout the experiment. X-ray examination of the long bones showed normal conditions. In both cases normal organs were found at autopsy.

Urine was collected during 24 hour periods from the 6th day and from the 12th day of the experiment for the determination of the excretion of ascorbic acid. In both cases 0.1 mg ascorbic acid was excreted per rat during 24 hours.

The ash and mineral contents of the femurs as well as the results of various laboratory examinations are given in table 2.

Summary of Results for Experiments 1, 2, and 3. From these experiments it may be concluded that excess of vitamin A in the form of highly purified vitamin A preparations dissolved in peanut oil, had the same injurious effect on rats as equivalent amounts of vitamin A in the form of whale liver oil concentrate.

In one experiment approximately 52,000 I. U. vitamin A daily, corresponding to approximately 800 I. U. vitamin A per gram body weight proved lethal in two cases after 11 days, and in one case at the end of 21 days. Similar or slightly smaller amounts of vitamin A in the form of whale liver oil concentrate proved lethal at the end of 26 to 30 days. The clinical symptoms observed in rats given highly purified vitamin A preparations were identical with those observed in rats given whale liver oil concentrate, as were the postmortem findings. Thus in both cases reduced weight gain, weakness, scruffiness, limping, fractures, alopecia and soreness of the skin around the eyes and mouth were observed as

Table 1.

Clinical Symptoms and Postmortem Findings in Rats Given Excess of Vitamin A in the Form of Whale Liver Oil, and Highly Purified Vitamin A Preparations. (Experiments 1, 2 and 3.)

Experiment no.	Conditions of experiments	Rat no.	Sex	Daily dose of vit. A, I. U.	Initial body weight, g	Duration of experiment, days	Clinical symptoms: Lethal result	Weight increase, g/day	Weakness	Scruffiness	Soreness of skin	Alopecia	Swelling of palpebrae	Exophtalmus	Limping	Fractures	Proteinuria	Postmortem findings: Subcutaneous hemorrhages	Visceral hemorrhages	Muscular hemorrhages	Enlarged adrenals	Fatty cut surface of liver	Fiery red lungs	Swollen visceral lymph glands	Histol. findings: Hyperemia	Red blood cells in space of Bowman's capsule	Sudanophil deposits in liver	Increased sudanophil deposits in adrenals	Degeneration of renal tubules
	Basal diet (control)	1	♂♀	20	64	24	0	3.3	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0
		2	♂♀	20	63	24	0	3.5	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
1.	Basal diet + excess of vit. A (whale liver oil)	1	♂	50000	61	27	+	1.0	0	+	+	0	0	0	+	+	} +	0	0	+	-	+	0	-	+	+	0	+	+	
		2	♀	50000	64	26	+	−0.3	+	+	0	+	0	0	+	+		0	+	+	+	-	+	-	-	-	-	-	-	-
		3	♂	50000	64	30	+	1.3	+	+	0	0	0	0	+	0		0	0	0	+	-	0	-	-	-	-	-	-	-
		4	♀	50000	65	26	+	−0.2	0	+	+	+	0	0	+	+		0	0	+	-	-	+	-	-	-	-	-	-	-
2.	Basal diet + excess of vit. A (acetate)	1	♀	52500	60	21	+	0.7	+	+	+	+	+	0	+	+	-	0	0	0	-	-	-	-	+	-	+	0	(+)
		2	♂	52500	61	11	+	−0.7	+	+	+	+	+	+	+	+	-	+	0	+	-	-	+	-	-	+	-	-	-
		3	♀	52500	61	11	+	−0.7	+	+	+	+	+	0	+	+	-	0	0	+	-	+	+	-	+	+	+	0	+
	Basal diet (control)	1	♀	20	87	28	0	2.6	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	-	-	-	-	-
		2	♀	20	93	33	0	2.1	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	-	-	-	-	-
3.	Basal diet + excess of vit. A (purified highly concentrated shark liver oil	1	♀	60000	80	38	0	0.3	+	+	+	+	+	0	+	+	-	0	0	0	-	+	+	+	+	-	+	+	0
		2	♀	60000	91	28	0	0.9	+	+	+	+	+	0	+	0	-	0	0	0	-	+	0	-	+	-	+	0	(+)
		3	♀	60000	84	42	0	−0.3	+	+	+	+	+	0	+	+	-	0	0	0	-	+	0	-	-	-	-	-	-
		4	♀	60000	88	42	0	−0.3	+	+	+	+	+	0	+	+	-	+	0	+	-	+	0	-	-	-	-	-	-

+ = positive findings. (+) = very slight, or uncertain findings. 0 = negative findings. - = not examined.

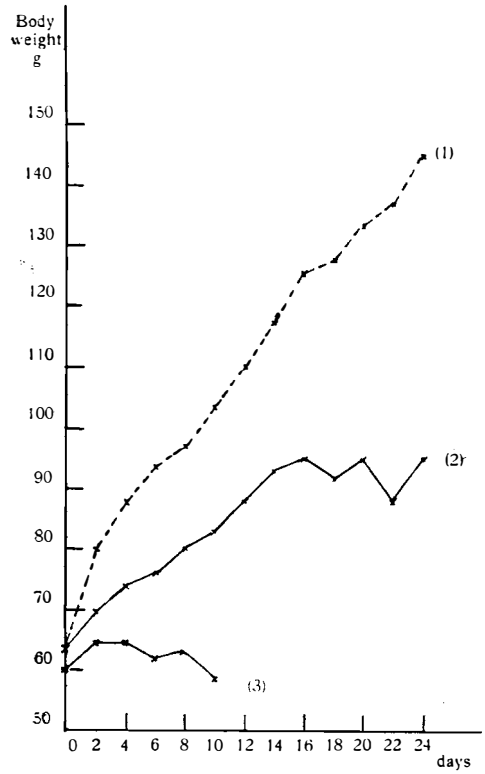
Table 2.

Results of Various Laboratory Examinations in Rats Given Excess of Vitamin A in the Form of Whale Liver Oil, and Highly Purified Vitamin A Preparations, Compared with Normal Rats. (Experiments 1, 2 and 3.)

Experiment no.	Conditions of experiments	Rat no.	Sex	Initial body weight, g.	Duration of experiment, days	I. U. vitamin A/day	Hb. %	Red blood cells, mill. pr. 1/1000 ml	Colour index.	Vitamin C in serum, mg/100 ml	Prothrombin time, seconds	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm.	Width of femur, mm.	Fractures	Hemorrhages
	Basal diet (control)	1	♀	64	24	20	102	-	-	0.54	-	53.5	36.3	17.4	28	2.3	0	0
1.	Basal diet + excess of vit. A (whale liver oil)	1	♂	61	27	50000	88	-	-	0.00	-	54.9	41.8	19.3	26	1.7	+	+
		2	♀	64	26	50000	-	-	-	-	-	51.7	36.8	20.6	24	1.7	+	+
		3	♂	64	30	50000	91	-	-	-	-	-	-	-	24	1.7	0	0
		4	♀	65	26	50000	-	-	-	-	-	48.1	33.6	22.6	24	1.7	+	+
		Mean:				50000	90	-	-	-	-	51.6	37.4	20.8	25	1.7	-	-
2.	Basal diet + excess of vit. A (acetate)	1	♀	60	21	52500	84	-	-	0.39	-	56.2	33.9	18.7	23	1.9	+	0
		2	♂	61	11	52500	-	-	-	-	-	49.6	23.6	21.6	-	-	+	+
		3	♀	61	11	52500	96	-	-	-	-	48.3	47.6	28.2	-	-	+	+
		Mean:				52500	90	-	-	-	-	51.4	35.0	22.8	-	-	-	-
	Basal diet (control)	1	♀	87	28	20	105	5.2	1.0	0.70	19.0	-	-	-	-	-	0	0
		2	♀	93	33	20	112	5.5	1.0	0.50	-	-	-	-	-	-	0	0
		Mean:				20	109	5.4	1.0	0.60	-	-	-	-	-	-	-	-
3.	Basal diet + excess of vit. A (purified highly concentrated shark liver oil)	1	♀	80	38	60000	82	-	-	0.18	-	58.2	31.8	18.6	25	2.0	+	0
		2	♀	91	28	60000	93	5.8	0.8	0.05	16.5	51.4	31.6	20.4	27	2.1	0	0
		3	♀	84	42	60000	58	4.3	0.7	-	24.0	57.8	29.2	17.5	26	2.0	+	0
		4	♀	88	42	60000	72	4.1	0.9	-	-	54.6	30.6	17.9	25	2.0	+	+
		Mean:				60000	76	4.7	0.8	0.12	20.3	55.5	30.8	18.6	26	2.0	-	-

well as hemorrhages, fatty appearance of the cut surface of the liver, fiery red colouring of the lungs, and histologically: hyperemia, red blood cells in the space of Bowman's capsule, deposits of sudanophil droplets in the liver, and increased deposits of sudanophil droplets in the adrenal cortex.

By examination of the blood a hypochromic anemia was detected in only two cases, although considerable hemorrhages were observed in several cases. The ascorbic acid content of the serum was considerably less than the average normal value. The total bases in the blood showed normal valus. The prothrombin time was increased in one case,



Graph No. 1. Average weight graphs for rats given excess of vitamin A in the form of whale liver oil (50,000 I.U. vit. A daily) (2), and vit. A acetate (52,500 I. U. vit. A daily) (3), compared with normal rats (1).

and the excreted amount of ascorbic acid in the urine during 24 hours was less in the hypervitaminotic rats compared with normal control rats.

Although there was considerable variation in the ash and mineral content of the bones, the average values were approximately the same as in the control animals.

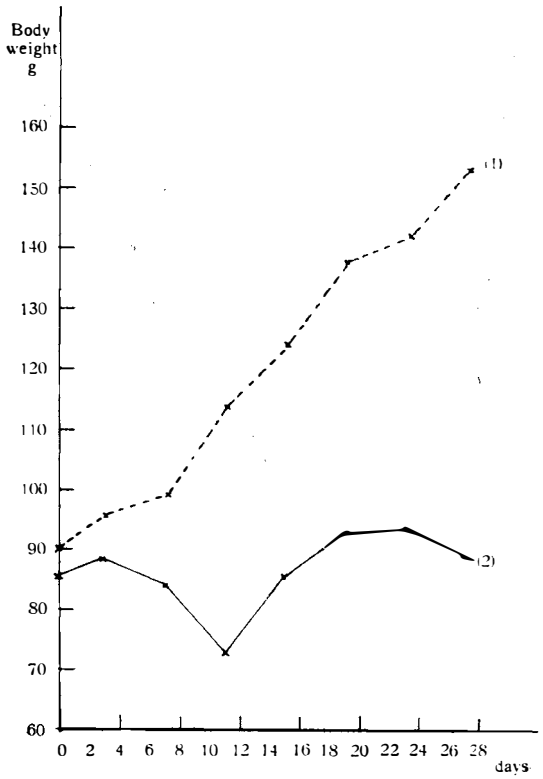
2. *The Effect of Various Fractions of Liver Oil Concentrates.*

The purpose of the following experiments was to examine the effect of various fractions of the same whale liver oil as used in the previous experiments on rats, — such as the fraction containing the vitamin A, as well as whale liver oil when the vitamin A had been destroyed, the "kitol", and the sterol fraction.

a) **Liver Oil Without Vitamin A.**

Experiment 4.

In a preliminary experiment, two rats with an initial body weight of approximately 100 g were given whale liver oil where practically all the vitamin A content had been destroyed by exposure to sunlight in open air during a period of 14 days. Each rat was given 6 drops of this oil by dropping pipet daily, and 12 drops mixed in the ordinary basal diet, during a period of 11 days, during which time the rats remained normal and free of any symptoms. The average daily weight gain was 4.3 grams.



Graph No. 2. Average weight graphs for rats given excess of vitamin A in the form of purified highly concentrated shark liver oil (2), compared with normal rats (1). (Experiment 3).

The rats were killed and examined in the usual manner. The postmortem examination revealed normal findings, and by microscopical examination of the liver, kidney, adrenals and pancreas of one of the rats, no pathological findings were revealed.

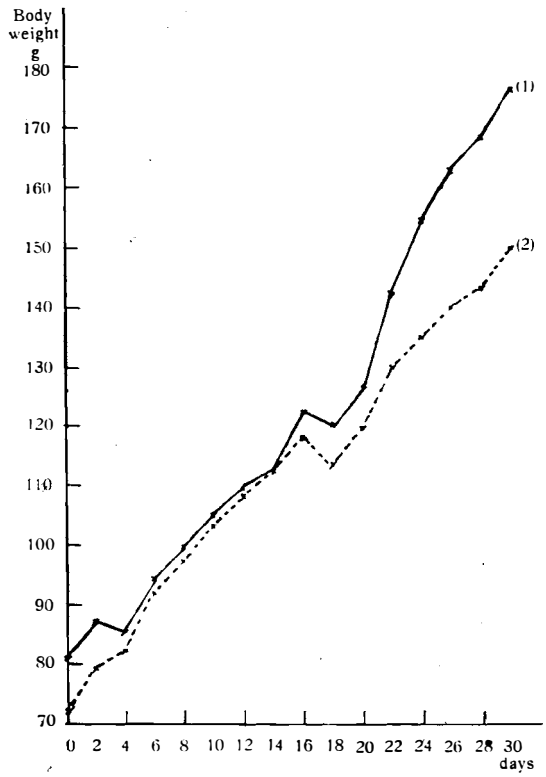
In one of the animals the hemoglobin was 108 %, and the ascorbic acid content of the serum was 0.60 mg per 100 ml, and in the second the hemoglobin was 102 %, and the ascorbic acid content 1.05 mg per 100 ml. In both cases the blood coagulated normally, and the serum colour was normal.

The ash content of the femur was 50.7 % calculated on a dry basis, and the calcium and phosphorus contents of the ash were 27.4 % and 18.0 % respectively.

Experiment 5.

In a further experiment, the effect of identical amounts of the same whale liver oil as used in experiment 1 was examined in rats, after the vitamin A had been completely destroyed. As the animals in this group were approximately the same age as the animals in experiment 1, and the amount of oil given was identical, the effect of the oil with and without vitamin A could thus be directly compared.

The vitamin A was destroyed by long lasting exposure to sunlight. The whale liver oil was transferred into large Petri's dishes, which were left exposed to sunlight in open air during a period of 6 weeks. At the end of this time the vitamin A was found to be destroyed, as judged by the antimony trichloride method. This oil was then given to two rats with initial body weights between 70 and 80 grams in amounts which would have yielded 50,000 I. U. vitamin A



Graph no. 3. Average weight graphs for rats given vitamin A-free whale liver oil (1), compared with normal rats (2). (Experiment 5.)

daily before the vitamin A was destroyed, i. e. ten drops. Six drops were given per rat mixed in the diet, and four drops by dropping pipet daily.

Another group of two rats of the same age was used as control, and were given the usual basal diet only. Both groups were given vitamins A and D in optimal growth doses. The rats were observed during a period of one month.

During the first 10 days of the experiment, the average daily weight gain in the control group was 3.1 grams, the entire weight gain at the end of this period being 43 % of the initial weight. During the same period the average daily weight gain in the group given the vitamin A-free whale liver oil was 2.9 grams (2.95 and 2.90 g in the individual rats), the entire weight gain at the end of the first 10 days being 38 % of the initial weight. (42.1 and 35.3 % in the two rats.)

During the second 10 day period of the experiment, the average daily weight gain in the control group was 1.60 g as against 2.06 g in the group given vitamin A-free whale liver oil.

During the final 10 days of the experiment, the average daily weight gain was 3.05 g for the control group, as against 5.04 g in the group given vitamin A-destroyed whale liver oil.

During the entire experiment, the average daily weight gain was 2.58 g for the control group, against 3.33 g for the group receiving vitamin A-destroyed whale liver oil. At the end of this period the weight gain was 107.5 % of the initial weight for the control group, as against 131.5 % for the test group.

The average weight graphs are given on page 34.

The group given the whale liver oil where the vitamin A had been destroyed, remained perfectly normal and free of any symptoms throughout the experiment,

while rats of the same age receiving the same amount of the same whale liver oil containing all its vitamin A invariably showed pronounced symptoms within 20 days.

The whale liver oil where the vitamin A had been destroyed, caused no epilation in the rats when given in the mentioned quantities, while whale liver oil containing all its vitamin A given in the same quantities invariably caused epilation in the rats when given orally.

During the first two weeks of the experiment, the weight curve for the mentioned group was practically identical with the control group, while the weight increase during the last two weeks of the experiment was higher in the group receiving the vitamin A-free whale liver oil, than in the control group.

The daily food consumption in the two groups was weighed during a period of 9 days from the 12th day of the experiment. During this period the average daily food consumption was 11.4 g for the control group, as against 12.3 grams in the group receiving the oil.

Thus the food consumption was greater in the group given the whale liver oil free of vitamin A, than in the control group, while rats given the same amount of whale liver oil containing all its vitamin A showed a much smaller food consumption even when the oil was given in the same manner (mixed in the diet, or by dropping pipet).

Table 3.

Weight Gain in Rats Given Whale Liver Oil where the Vitamin A had been Destroyed, Compared with the Control Group. (Experiment 5.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Weight gain, average			
				0—10 days g/day	10—20 days g/day	20—30 days g/day	0—30 days g/day
Basal diet (control)	1	♀	67.0	3.1	1.6	3.1	2.6
	2	♀	77.0				
Basal diet + vit. A-free whale liver oil	1	♀	70.0	2.9	2.1	5.0	3.3
	2	♂	82.0				

X-ray examination of the long bones at the conclusion of the experiment, showed normal conditions in both groups (ill. 13).

At the end of one month the animals in both groups were killed and examined in the usual manner. In all cases normal organs were found by macroscopical post-mortem examination, except for small abscesses in the left kidney in one of the control rats, and an enlarged spleen and fatty appearance of the cut surface of the liver in the two rats given excess of vitamin A-free whale liver oil.

Microscopical examination of both groups revealed normal findings in the ovaries, testes, intestines, liver, pancreas, kidneys, adrenals, lungs, spleen, heart and bones.

In one of the control rats, as well as both rats given the whale liver oil where the vitamin A had been destroyed, the following organs were stained by sudan III: spleen, liver (ill. 29), heart, kidney, adrenal, lung, intestines, pancreas and thymus. Except for in the adrenal cortex (ill. 39, 41), no deposits of sudanophil droplets were detected in any of these rats.

The results of blood examinations are given in table 4. From this table it is seen that the hemoglobin values are not affected by whale liver oil free of vitamin A.

Table 4.

Hemoglobin, and Vitamin A Content in Blood of Rats Given Whale Liver Oil Free of Vitamin A, Compared with Normal Control Rats. (Experiment 5.)

Conditions of experiment	Rat no.	Sex	Hb 0/0	Vit. A I. U./g serum
Basal diet (control)	1	♀	102	< 2
	2	♀	104	
Basal diet + vit. A-free whale liver oil	1	♀	104	< 1.7
	2	♂	107	

The average vitamin A content of the livers was 125 I. U. vit. A/g liver for the control, and 800 I. U. vit. A for the group receiving whale liver oil, when the vitamin A had been destroyed.

The ash content of the femurs and the mineral content of the ash are shown in table 5. From this table it appears that there is no reduction of the ash and mineral content of the bones in rats given whale liver oil free of vitamin A.

Table 5.

Ash and Mineral Contents of the Femurs in Rats Given Whale Liver Oil Free of Vitamin A, Compared with Normal Control Rat. (Experiment 5.)

Conditions of experiment	Ash, 0/0 of dry bone	Ca, 0/0 of ash	P, 0/0 of ash
Basal diet	48.7	32.3	18.8
Basal diet + whale liver oil free of vit. A	53 8	30.3	18.0

From these experiments it may be concluded that the whale liver oil has no injurious effect on rats when the vitamin A content of the oil has been destroyed.

b) **Purified Liver Oil Concentrate.**

Experiment 6.

A sample of saponified whale liver oil containing 637,000 I. U. vit. A/g was freed of sterols and "kitol" (see page 37). This purified whale liver oil concentrate was given to a group of three young rats with initial body weights of approximately 50 grams, in amounts of three drops daily, during a period of 26 days.

Slight eye symptoms were observed at the end of 5 days. Soreness and alopecia was observed at the end of 7—10 days in all rats in this group, and they appeared weak and scruffy from the 12th day. The average weight gain throughout the experiment was less than normal.

At the end of 26 days, the rats were killed and examined in the usual manner. By postmortem examination no significant pathological findings were revealed by macroscopical examination, apart from the clinical symptoms already described.

The results of blood examinations are given in table 6, from which it is observed that the ascorbic content of the serum is considerably less than normal in rats given purified whale liver oil concentrate, but the hemoglobin values were within the normal range.

Table 6.

The Results of Blood Examinations in Rats Given Purified Whale Liver Oil Concentrate. (Experiment 6.)

Rat no.	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	asc. acid in serum, mg/100 ml.
1	97	5.0 mill.	1.0	0.260
2	89	5.16 mill.	0.8	0.175
3	88	5.10 mill.	0.9	0.320

By X-ray examination of the long bones the shafts appeared abnormally thin.

The ash and mineral content of the femurs is given in table 7. The values are normal for rats of this age.

Table 7.

Ash and Mineral Contents of the Femurs in Rats Given Purified Whale Liver Oil Concentrate. (Experiment 6.)

Rat no.	Ash, % of dry bone	Ca, % of ash	P, % of ash
1	53.3	34.2	18.7
2	55.3	31.0	17.7
3	55.7	-	-
Mean:	54.8	32.6	18.2

c) **"Kitol".**

Simultaneously, the same amount of "kitol" [1] (3 drops daily) was given to a rat with the same initial body weight as the rats in the forementioned experiment, during a period of 26 days. No ill effect was observed in the rat. The weight gain was normal (3.3 grams daily), and the rat was free of any symptoms.

By postmortem examination normal organs were found, and by microscopical examination of the liver, kidney, adrenal, spleen, pancreas, ovary, uterus, tibia and teeth, no pathological findings were detected.

The hemoglobin was 112 %, red blood cells 4.8 millions per 1/1000 ml, and colour index 1.1. The ascorbic acid content of the serum was 0.55 mg/100 ml.

The ash content of the femur was 54.0 % calculated on a dry basis.

[1] "Kitol" — a dihydric alcohol found in considerable quantities in whale liver oil, — transformed into vitamin A upon heating to temperatures above 200° C (Embree, N. D., Shantz, E. M. (1943)), separated by exhaustive extraction with 83 % alcohol, as described by Kringstad & Lie (1941).

d) **Sterols.**

Experiment 7.

Finnally a sample of sterols, isolated from the same whale liver oil, was given to a group of two rats of the same age as the previous group in amounts of 100 mg per rat daily, during a period of four weeks. This amount of sterols, which was mixed in the ordinary basal diet daily, corresponded approximately to the amount of sterols present in the daily dose of whale oil given to each of the rats in experiment 1.

Although the weight increase in one of the rats was less than normal, both rats appeared normal throughout the experiment. They were killed in the usual manner at the end of one month, and postmortem examination revealed normal findings. By histological examination of the liver, adrenals, pancreas, spleen, kidneys, ovaries, bones and teeth, no pathological findings were made. No sudanophil deposits were observed in the liver stained by sudan III, and in the adrenals normal sudanophil droplets were observed in the cortex.

The hemoglobin was 104 and 105 %, and the ascorbic acid content of the serum was 0.58 and 0.40 mg per 100 ml serum.

The ash content of the femurs was 66.1 % calculated on a dry basis, and the calcium and phosphorus contents of the ash were 36.2 % and 17.1 % respectively.

Summary of Results from Experiments 4—7. From these experiments it is evident that the toxic factor of whale liver oil is identical with vitamin A, and that none of the other fractions of the whale liver oil concentrate were responsible for the toxic effect on rats.

3. *Comparison of the Effect of Different Methods for Oral Administration of Vitamin A-Oil.*

Experiment 8.

For the purpose of comparing the effect of excess of vitamin A when given to the rats mixed in the diet, and when given by a catheter or dropping pipet, the two different methods of feeding were applied to two separate groups of male adult rats, a third group of rats of the same weight being used as control and given basal diet only.

The first group, consisting of six rats with an average initial body weight of approximately 200 grams, was given 6 drops of whale liver oil concentrate daily (30,000 I. U. vit. A), mixed in the usual basal diet. The diet mixture was prepared fresh daily. It was found that this amount of oil produced a suitable food mixture. Another 4 drops of the same oil (20,000 I. U. vit. A) were given to the rats daily by a specially constructed metal catheter in addition, making a total dosage of 50,000 I. U. vit. A per rat per day, or approximately 260 I. U. vit. A/g body weight daily. At the end of 12 days the dosage by catheter was increased to 12 drops, making a total dose of 90,000 I. Ut. vit. A daily or 480 I. U./g body weight, for the purpose of producing more pronounced symptoms.

The second group, consisting of six rats with the same average initial body weight, were given 10 drops of the same whale liver oil concentrate, corresponding to 50,000 I. U. vit. A daily, or an average of 250 I. U. vit. A/g body weight by catheter, in addition to the ordinary adequate basal diet. The vitamin A dose was

increased after the first 10 days to 90,000 I. U. vit. A daily, corresponding to an average of 390 I. U. vit. A/g body weight.

For the purpose of determining how much of the given dose of vitamin A was lost through feces, the feces was collected during a 24 hour period from all three groups from the 14th to the 15th day of the experiment. The results are given in table 8.

Table 8.

Loss of Vitamin A Through Feces. (Experiment 8.)

Conditions of experiment	Daily gross dose of vit. A. I. U.	Feces in 24 hours per rat, g	% fat in feces	I. U. vit. A/g fat	Total I.U. vit. A in feces	Loss of vit. A through feces in % of gross dose
Basal diet (control)	20	4.5	6.5	0	0	0
Basal diet + excess of vit. A mixed in diet + by catheter	90,000	4.2	10.0	84,000	36,540	40.6
Basal diet + excess of vit. A by catheter only	90,000	4.7	13.1	72,000	44,460	49.4

In the first group, 4.2 grams feces was extracted per rat in 24 hours, containing 10 % fat, which yielded 84,000 I. U. vit. A/g. The total amount of vitamin A excreted in 24 hours was 36,540 I. U. per rat, — i. e. 40.6 % of the gross dose of vitamin A was lost through feces when given in the described manner, — both mixed in the diet and given by catheter.

In the second group the excreted amount of feces in 24 hours was 4.7 grams. The fat content was 13.1 % with a vitamin A potency of 72,000 I. U./g. The total amount of vitamin A excreted during 24 hours was 44,460 I. U. per rat, of a total gross dose of 90,000 I. U., i. e. 49.4 % of the gross dose of vitamin A was lost through feces. No vitamin A was detected in the feces from the control group kept on the basal diet.

The stated figures must be regarded as minimum figures, as some deterioration of the vitamin A content in the feces might have taken place during the 24 hour period.

Although these figures only refer to one 24 hour period in the middle of the experiment, they may be taken as evidence of the fact that of the given gross dose of vitamin A, only 50—60 % at the most was absorbed by the rat, and that the loss of vitamin A through feces appears to be greater when the oil is given by catheter or dropping pipet than when mixed in the basal diet.

While the control group remained normal and free of any symptoms throughout the experiment, except for a short-lasting soreness of one of the eyes in one case, all rats in the first group, except for two, lost weight during the experiment. In the second group the average daily weight gain was 0.1 grams as against 0.9 for the control group. The average weight graphs compared with the control are shown on page 42.

Examination of the urine from the two groups given excess of vitamin A revealed proteinuria and hematuria in both cases.

Urine was collected from the rats in the first group during a 24 hour period, from the 13th—14th day of the experiment. Three ml was excreted per rat. The

Heller's test was positive, and the acetic acid test was strongly positive, as was the benzidine test. By microscopical examination of the centrifuged urine, many red blood cells were seen per field of vision (proteinuria and hematuria).

From the second group urine was collected during a 24 hour period from the 39th—40th day of the experiment. The excreted amount of urine per rat in 24 hours was 1.5 ml. The Heller's test and the boiling test were faintly positive, the benzidine test was strongly positive after 10 seconds, and microscopical examination of the centrifuged urine revealed many red blood cells per field of vision. Haine's test for sugar was negative, as was Schlesinger's test (dilution: 1/10).

The urine was again collected during a second 24 hour period from the 41st to the 42nd day of the experiment for the determination of ascorbic acid. The excreted amount was 2 ml per rat in 24 hours, and the excreted amount of ascorbic acid was 0.0 mg per rat in 24 hours.

In the first group the following symptoms were observed: In one of the rats, swelling of the palpebrae occurred on the third day, and fracture on the 24th day. In the second rat, the eye symptoms also occurred on the third day, limping on the 10th, and fracture on the 44th day. In the third rat, soreness around the nose and limping was observed on the 15th day, and fracture the following day. In the 4th rat, soreness around the eyes occurred on the third day, swelling of the palpebrae, which lasted a month, on the 6th day, — weakness from the 39th day and fracture on the 44th day. In the 5th rat, soreness around the eyes was observed on the 24th day. Otherwise no distinct clinical symptoms were observed. In the final rat, fracture occurred on the 28th day.

The rats were killed and examined in the usual manner from 16 to 48 days after the commencement of the experiment. The blood coagulated within 5 minutes in all cases. In one case, where there were particularly large hemorrhages around the fractures, anemia was observed (hemoglobin: 58 %). Otherwise the hemoglobin was between 84 and 98 %, although there were considerable hemorrhages around the fractures also in these cases.

By postmortem examination, no pathological findings were revealed in one case, while in the remaining five rats, hyperemia and large hemorrhages around the fractures were revealed in all cases. Apart from this, no subcutaneous hemorrhages were detected. Enlarged visceral lymph glands were observed in three cases. The liver appeared fatty on the cut surface in three cases.

In two of the rats in this group, the liver, kidneys, adrenals, pancreas, spleen, intestines, testes, lungs, heart and bones were examined histologically. Hyperemia was detected in all cases. In the kidney there was slight degeneration of the renal tubules, some of which were filled with remnants of cells, and free blood in some cases. By sudan III staining, very dense deposits of sudanophil droplets were observed throughout the liver, both in and between the liver cells, particularly in the Kupffer cells, some of which appeared swollen. In the adrenal, abnormally dense deposits of sudanophil droplets were seen in the zona glomerulosa of the cortex (see ill. 42). By sudan staining of the heart, kidney and spleen, no sudanophil deposits were observed, while sudanophil droplets were detected in the mucous membrane of the intestines. Microscopical examination of the bones (tibia) revealed hyperemia and great irregularity og bone structure. In the other organs, no significant pathological findings were revealed.

In the second group receiving the whale liver oil concentrate by catheter, the following symptoms were observed: One of the rats (no. 5) died at the end of 6 days from pneumonia, at which time soreness and alopecia were observed around the nose and eyes. The animal appeared to be in good condition, and there were no subcutaneous hemorrhages or fractures. There was general hyperemia,

however, and hemorrhages in the intestines. The thymus appeared enlarged. There were small hemorrhages in the meninges. Microscopical examination of the lungs showed alveoli filled with exudate containing red blood cells and leucocytes, and there were perivascular and peri-alveolar leucocytic infiltrations (bronchopneumonia). Slight deposits of sudanophil droplets were detected in the liver, and dense deposits of sudanophil droplets were seen in the adrenal cortex. In the kidneys, pancreas, testes and heart no significant pathological findings were made, while large amounts of pigment were detected in the spleen.

Another rat (no. 4) showed fracture of the left fore leg on the 13th day, at which time there was soreness around the mouth. By postmortem examination detachments were seen of the proximal epiphyses of both tibiae. The liver was fatty on the cut surface, and accessory adrenals were found. There were no subcutaneous or visceral hemorrhages, except around the fracture of the left scapula. By microscopical examination, hyperemia was detected in the kidneys, and large deposits of sudanophil droplets were observed in the liver and abnormally dense deposits in the periphery of the adrenal cortex. No significant pathological findings were made in the spleen, testes, pancreas, intestines, lungs or the heart.

Of the surviving rats, all appeared weak and scruffy on the 20th day. Rat no. 1 suffered from diarrhea shortly after the commencement of the experiment, swelling (oedema) of the palpebrae on the 4th day, possibly exophtalmus on the 8th day, as well as swelling of the forehead and limping on the 35th day, without any fractures being detected. Rat no. 2 limped on the 12th day, and fractures with hemorrhages of the left hind leg were observed on the 20th day. Rat no. 6 showed exophtalmus on the 8th day.

Towards the conclusion of the experiment these last mentioned rats were practically free of clinical symptoms.

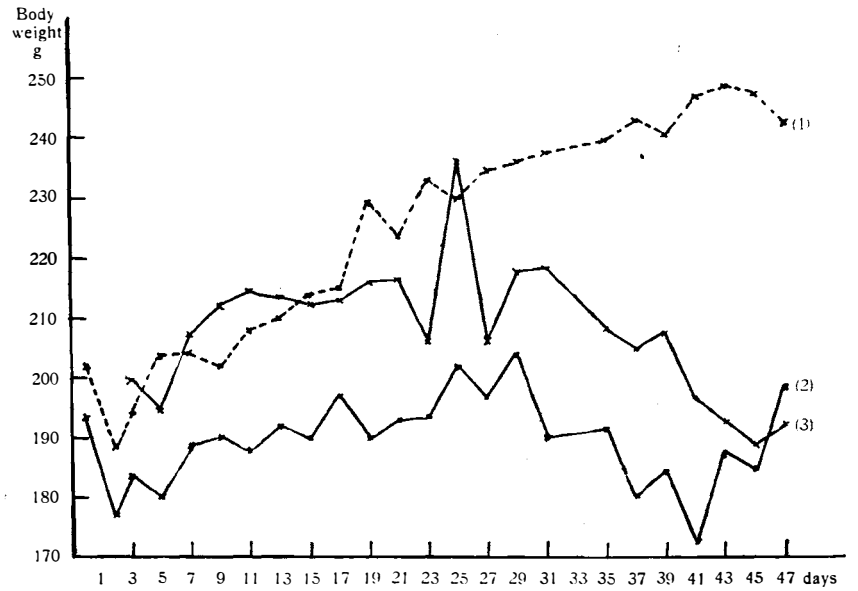
They were killed and examined in the usual manner from 1—4 hours after the last dose of vitamin A had been given. At autopsy, no significant pathological findings were revealed in the internal organs, except that the liver was fatty on the cut surface. The rats were in relatively good condition.

The blood coagulated normally in all cases. There was distinct lipemia in the rats killed 1 and 2 hours after the last dose of vitamin A, but only very faintly in the two killed 3 and 4 hours after the dosage. In no case was the serum colour increased.

X-ray examination revealed fracture of both tibia and fibula in the left hind leg of rat no. 2, as well as the described epiphyseal deformities and fracture of the scapula in rat no. 4 (see ill. 17).

The results of the various laboratory tests are given in tables 10 and 11. For practical reasons the vitamin A content of the serum in these rats is considered in a later chapter.

In rats nos. 1 and 6 the liver, kidneys, adrenals, pancreas, spleen, testes and lungs were examined histologically. Marked hyperemia was observed in all cases. Scattered red blood cells were seen outside the capillaries in the kidney in one of the rats, and by Turnbull's staining, signs of small old hemorrhages were detected. Some of the convoluted and collecting tubules contained amorphous masses, and deposits of calcium, stained dark blue with hematoxylin-eosin, and black with Kossa's staining (see ill. 35). No pathological findings were revealed in the pancreas, spleen or testes. Massive deposits of sudanophil droplets were seen in the liver, particularly between the liver cells (Kupffer's cells) (see ill. 28). In the adrenal, defined strips of strongly stained dense deposits of sudanophil droplets were seen in the zona glomerulosa of the cortex in one case, and in another case the entire cortex was filled with very dense deposits of sudanophil droplets (see



Graph no. 4. Average weight graphs for rats given excess of vitamin A (50—90,000 I.U. vit. A daily) mixed in the basal diet and by catheter (2), and by catheter only (3), compared with normal rats (1).

ill. 40). No fatty degeneration was observed in the parenchyma of the kidney and spleen, while massive sudanophil deposits were detected in the alveolar walls, as well as in the alveolar lumen of the lung in patches (se ill. 37).

In the normal control rats, no pathological findings were detected in the mentioned internal organs or the bones. When stained by sudan III, no sudanophil deposits were detected in the liver, spleen, heart, kidney, lungs or intestines. The normal sudanophil deposits were observed in the adrenal cortex.

Summary of Results from Experiment 8. From this experiment it appears that the same gross dose of vitamin A proved slightly more injurious when partly mixed in the basal diet than when given entirely by catheter. In the latter case a larger proportion (approximately 20 % more) of the gross dose of vitamin A was lost through feces, which possibly might account for this difference in the observed effect of the same gross dose of vitamin A when administered in the two different ways.

Of the clinical symptoms (see table 9), reduced weight gain, weakness, scruffiness, soreness around the eyes and mouth, swelling of the palpebrae, exophtalmus, limping and fractures occurred in both cases, but the symptoms were more pronounced in the group given excess of vitamin A both mixed in the basal diet and by catheter, than in the group given the same dose of vitamin A exclusively by catheter. In both groups

Table 9.

Clinical Symptoms and Postmortem Findings in Rats Given Excess of Vitamin A Mixed in Basal Diet, and by Catheter. (Experiment 8.)

Conditions of experiment	Rat no.	Daily dose of vit. A, I. U.	Initial body weight, g	Duration of experiment, days	Lethal result	Reduced weight gain	Weakness	Scruffiness	Soreness of skin	Alopecia	Swelling of palpebrae	Exophtalmus	Limping	Fractures	Proteinuria	Hematuria	Pneumonia	Subcutaneous hemorrhages	Visceral "	Muscular "	Fatty cut surface of liver	Swollen lymph glands	Detachment of epiphyses	Hyperemia	Red blood cells outside capillaries	Red blood cells in the space of Bowman's capsule	Sudanophil deposits in liver	Increased sudanophil deposits in adrenal cortex	Degeneration of renal tubules
Basal diet (control)	1	20	233	50	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	20	169	50	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0
Basal diet + excess of vit. A given mixed in basal diet, and by catheter	1	50000—90000	183	24	0	+	0	0	0	0	+	0	-	+			0	÷	0	+	+	+	0	0	+	+	-	+	-	-
	2	»	202	44	0	+	0	0	+	0	+	+	+	+			0	+	0	+	+	0	0	0	+	(+)	(+)	+	+	+
	3	»	178	16	0	+	0	+	+	0	0	0	+	+			0	+	0	+	0	0	0	0	+	(+)	(+)	+	+	+
	4	»	176	45	0	+	+	0	+	0	+	0	+	+			0	-	0	+	0	+	0	0	+	-	-	-	-	-
	5	»	220	48	0	+	0	0	+	0	0	0	0	0			0	0	0	0	0	0	0	0	+	-	-	-	-	-
	6	»	204	29	0	+	0	0	0	0	0	0	-	+			0	+	0	+	+	+	0	0	+	-	-	-	-	-
Basal diet + excess of vit. A given by catheter only	1	50000—90000	203	46	0	+	+	+	0	0	+	(+)	+	-			0	0	0	0	+	0	0	+	0	0	+	-	(+)	
	2	»	223	46	0	+	+	+	0	0	+	0	+	+			0	0	0	(+)	+	0	0	-	-	-	-	-	-	
	3	»	150	46	0	+	+	+	0	0	0	0	0	0			0	0	0	0	+	0	0	-	-	-	-	-	-	
	4	»	197	13	0	+	0	0	+	0	0	0	-	+			0	(+)	0	(+)	+	0	0	+	+	0	0	+	0	
	5	»	211	6	+	+	0	0	+	+	0	0	0	0			+	0	+	0	0	0	0	+	+	0	+	+	0	
	6	»	216	46	0	+	+	+	0	0	0	+	0	0			0	0	0	0	0	0	0	+	+	+	0	0	0	

Table 10.

Results of Various Laboratory Examinations in Adult Rats Given Excess of Vitamin A (250—480 I. U. Vit. A/g Body Weight), Compared with Normal Rats. (Experiment 8.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Blood: Hb %	Blood: mg ascorbic acid /100 ml serum	Blood: Total base in serum, milliequiv/l	Blood: Urea in blood, mg/100 ml	Liver: Liver weight, g	Liver: Fat in whole liver, g	Liver: I.U. vit. A/g fat	Liver: I. U. vit. A/g liver	Liver: Total I. U. vit. A in whole liver	Liver: Ascorbic acid in liver, mg/100 g	Adrenal: Ascorbic acid in adrenal, mg/100 g	Adrenal: Weight of left adrenal, g	Adrenal: Weight of left adrenal in % of body weight
Basal diet (control)	1	♂	233	50	104	0.70	-	-	-	-	-	-		25	125	0.0206	0.008
	2	♂	169	50	102	0.56	-	-	-	-	-	-	-	22	103	0.0218	0.010
	Mean:		-	-	103	0.63	-	-	-	-	-	-	-	24	114	0.0212	0 009
Excess of vit. A	1	♂	183	24	93	-	-	-	4.7	0 284	303000	18308	86052	-	-	0.0244	0.015
(50000—90000 I. U.	2	♂	202	44	58*	0.37	-	-	6.9	0.444	226000	14540	100344	8	69	0.0358	0.020
/day 260—480 I.U.	3	♂	178	16	98	-	-	-	6.8	0.284	209000	8728	59356	-	-	0.0216	0.012
/g body weight)	4	♂	176	45	84	0.49	151	-	6.0	0.366	223000	13603	81618	11	90	0.0334	0.013
mixed in basal diet	5	♂	220	48	93	0.32	-	58.7	-	-	-	-	-	21	20	0 0370	0.020
and by catheter	6	♂	204	29	93	-	-	-	7.9	0.188	22000	522	4131	-	-	0 0271	0.014
	Mean:		-	-	87	0.39	-	-	6.5	0.315	196600	11140	66300	13	60	0.0299	0.016
Excess of vit. A	1	♂	203	46	104	0.45	150	-	-	-	-	-	-	20	49	0 0410	0.019
(50000—90000 I.U.	2	♂	223	46	93	0.46	148	-	-	-	-	-	-	17	40	0.0288	0.013
/day, 200—390 I.U.	3	♂	150	46	93	0 41	150	-	-	-	-	-	-	19	72	0.0347	0.020
/g body weight)	4	♂	197	13	96	-	-	-	-	-	-	5698	-	-	-	0.0187	0.008
given by catheter	5	♂	211	6	-	-	-	-	-	-	-	-	-	-	-	-	-
only	6	♂	216	46	93	0.20	-	-	-	-	-	-	-	18	59	0.0338	0.015
	Mean:		-	-	96	0.37	149	-	-	-	-	-	-	19	55	0.0314	0.015

* — large muscular hemorrhage.

Table 11.

Ash and Mineral Contents of Femurs in Adult Rats Given Excess of Vitamin A Mixed in the Basal Diet, and by Catheter, Compared with Normal Rats. (Experiment 8.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Basal diet (control)	1	♂	233	50	55.3	33.7	16.5	30	2.5	0
	2	♂	169	50	55.2	31.8	17.0	26	2.5	0
	Mean:		-	-	55.3	32.8	16.8	28	2.5	-
Basal diet + excess of vit. A (50000—90000 I. U./day, 260—480 I.U./g body weight) mixed in basal diet, and by catheter	1	♂	183	24	57.5	41.2	16.8	-	-	+
	2	♂	202	44	57.9	35.0	16.4	25	2.2	+
	3	♂	178	16	55.1	35.3	15.7	30	2.0	+
	4	♂	176	45	59.2	31.3	17.5	32	2.0	+
	5	♂	220	48	59.6	34.1	15.7	35	2.5	0
	6	♂	204	29	54.9	33.3	16.4	-	-	+
	Mean:		-	-	57.3	35.0	16.4	31	2.2	-
Basal diet + excess of vit. A (50000—90000 I.U./day 250—390 I.U. /g body weight) given by catheter only	1	♂	203	46	56.7	32.3	16.5	35	2.5	0
	2	♂	223	46	57.2	29.7	15.5	-	-	+
	3	♂	150	46	59.1	34.7	17.2	30	2.0	0
	4	♂	197	13	54.7	34.8	16.9	-	-	+
	5	♂	211	6	55.6	32.8	18.0	-	-	0
	6	♂	216	46	58.4	33.8	16.0	-	-	0
	Mean:		-	-	57.0	33.0	16.7	33	2.3	-

hematuria and proteinuria was observed. Postmortem examination revealed hyperemia in the internal organs, muscular hemorrhages around the fractures, and fatty appearance of the cut surface of the liver in both cases.

In addition to this, swollen visceral lymph glands were observed in the group given excess of vitamin A mixed in the basal diet and by catheter, and visceral hemorrhages and detachment of the tibial epiphysis in the group given the same dose of vitamin A by catheter only.

Microscopical examination of the internal organs revealed similar findings in both groups.

From table 10 it is observed that although the hemoglobin was generally less in the hypervitaminotic rats than in the normal control rats, distinct anemia was detected only in one hypervitaminotic rat, in which case large muscular hemorrhages were observed.

The ascorbic acid content of the blood, adrenals and liver, was in all cases less in the hypervitaminotic rats than in the normal rats in this experiment. The total base in the blood was normal. The weight of the adrenals was generally higher in hypervitaminotic rats than in the normal rats.

The ash and mineral content of the bones (see table 11) was practically identical in hypervitaminotic rats and in normal control rats.

4. *The Effect of Excess of Vitamin A by Subcutaneous Injection.*

Lethal effect of single massive doses of vitamin A given by subcutaneous injection to rats, has been reported by several workers, (Takahashi et al. 1925, Matsuoka, 1934) who found that this treatment usually caused death with cramps in their animals, while other workers (Moore and Wang, 1945) failed to produce fatal result by subcutaneous injection of excess of vitamin A. It was therefore considered desirable in connection with the present investigations to compare the effect of equivalent massive doses of vitamin A given by subcutaneous injection, with oral administration in rats.

a) **Subcutaneous Injection of Liver Oils.**

Experiment 9.

The purpose of this experiment was to study the effect on rats of subcutaneous injection of large doses of vitamin A in the form of bear liver oil or whale liver oil concentrate.

Two normal female rats with initial body weights between 70 and 90 grams were daily injected subcutaneously with the oil by a very fine needle. Great care was taken that needle should not injure any blood vessel causing the oil to be introduced into the blood stream with the possible formation of fat embolus.

To start with 0.25 g bear liver oil was injected daily. After a few days the bear liver oil was exchanged for whale liver oil concentrate which was more applicable for this purpose. The injected doses of vitamin A varied from approximately 50,000 I. U. to 130,000 I. U. per rat per day in each injection. Great care was taken that the oil, which was injected deep into the subcutaneous tissue, should not escape through the opening in the skin made by the needle. The places of the injection varied from day to day. It was quite obvious that the oil was not easily absorbed as large lumps of unabsorbed oil could be palpated several days after the injection.

No immediate result could be seen on the animal after the injection. At the end of two days it was found that the hair was very loose all over the body, and could easily be removed in large tufts. The following day marked loss of hair occurred at the places of injection. At the end of five days from the beginning of the experiment one of the rats died, probably as the result of infection, after a total injection of 285,000 I. U. vitamin A.

By postmortem examination, marked alopecia was found over the whole abdomen and the places where the oil had been injected. It was found that a large part of the injected oil was still unabsorbed. There was a marked capillary reaction around the places of injection, particularly over the abdomen where

large dilated subcutaneous vessels were seen. A considerable hemorrhage was found over the right clavicula on the neck. The liver was very dark in colour and coagulated blood was found at the basis of the heart. The thymus was very small and atrophic. There were distinct signs of peritonitis. The urine bladder was punctured by a small needle and a sample of urine was collected in which Heller's test was positive.

On the tenth day, several abscesses from which pus was discharged were found in the skin on the back and abdomen in the surviving rat. So far no definite symptoms of hypervitaminosis A had been observed.

On the thirteenth day the rat had exophtalmus and the general condition was very poor. Apart from this no distinct sign of hypervitaminosis A could be detected by clinical examination. At that time the pus secretion had ceased, the wounds were healed, and the loss of hair was less marked.

On the twenty-second day soreness of the left eye was observed, and a swelling of the eye occurred two days later. These symptoms persisted for several days. There was also distinct limping in both hind legs, although X-ray examination on the thirtieth day revealed no fracture.

At the end of thirty-four days the rat was killed and examined after having received more than two million I. U. vitamin A. The average daily weight increase throughout the experiment was 1.5 grams.

The blood coagulated normally. The hemoglobin was 77 %, and the sedimentation reaction was 5 mm.

There were no marked eye symptoms, but some degree of alopecia, as well as marked hyperemia. Large yellow deposists of unabsorbed fat were found in the subcutaneous tissue over the abdomen and chest. Both the spleen, liver and the kidneys appeared enlarged.

b) **Subcutaneous Injection of Vitamin A Emulsion.**

Experiment 10.

Vitamin A in an emulsive form was injected subcutaneously in two female rats, with initial body weights of approximately 100 grams. This source of vitamin A was found to be more readily absorbed than the whale liver oil. 20,000 I .U. vitamin A was injected every other day, corresponding to approximately 100—80 I. U. vit. A/g body weight daily. The rats were injected with this dose of vitamin A during a period of 19 days, at the end of which the injections were discontinued and the rats were observed during a further period of 14 days.

The average daily weight gain during the first period was 1.7 grams, and during the second period, when no further doses of vitamin A were given, it was 0.8 grams.

In both cases marked eye symptoms occurred after 6 to 10 days. There was soreness around the eyes, and both eyes were practically closed as a result of marked swelling of the palpebrae. In one of the rats, these eye symptoms persisted throughout the experiment, even after the injection with vitamin A was discontinued. In one of the rats, infection occurred at the places of injection, and towards the conclusion of the experiment marked general alopecia was observed. In the second rat, a large tumour was observed on the left fore leg on the ninth day of the experiment, and by closer examination it was found to be a hematoma following a large subcutaneous hemorrhage.

X-ray examination revealed no fractures in any of the rats.

The rats were killed and examined in the usual manner. In one of the rats the hemoglobin was 89 %, and the ascorbic acid content of the serum was 0.275 mg per 100 ml; in the second rat the hemoglobin was 102 %.

In the first rat several tumours were found in the subcutaneous tissue at the place of injection. Apart from this no significant pathological findings were made. The vitamin A content of the liver was 5,320 I. U./g. Histologically marked hyperemia and vacuoles were observed in the pulp of the teeth. In the pancreas, uterus and spleen no pathological findings were made. In the kidneys, hyperemia, free blood in the space of Bowman's capsule, as well as slight degeneration of the tubules with deposits of a calcium-like substance were detected. By sudan III staining of the liver, large deposits of sudanophil droplets were seen throughout the organs, both in and between the liver cells. Abnormally dense deposits of sudanophil droplets were seen throughout the adrenal cortex, particularly in the periphery.

The ash content of the femur was 57.1 % calculated on a dry basis, and the calcium and phosphorus contents of the ash were 35.8 % and 17.6 % respectively.

In the second rat, which suffered from diarrhea, there was general hyperemia. There were signs of peritonitis, and adhesions between the stomach, kidneys and spleen. The thymus appeared enlarged, and attached to this several formations, which resembled swollen lymph glands, were seen. The abdominal cavity was filled with a serous fluid. The liver appeared fatty on the cut surface.

c) **Subcutaneous Injection of Single Massive Doses of Vitamin A.**

Experiment 11.

In a final experiment, one adult rat was given 1 million I. U. vitamin A in the form of a highly concentrated whale liver oil (713,000 I. U./g) in a single subcutaneous injection. The same quantity of peanut oil was injected subcutaneously in another adult rat for control purposes. The rats were then given ordinary basal diet and observed during a period of 7—11 days.

There was no immediate reaction to this treatment, and both rats remained free of any symptoms. They were killed and examined in the usual manner, and by postmortem examination normal organs were found in both cases. The injected peanut oil was completely absorbed, while the majority of the whale liver oil was still unabsorbed. It was encapsuled in a bag of fibrous tissue, which was attached to the skin. The bag was found to contain approximately 4/5 of the injected oil.

The hemoglobin was 102 % — as against 111 % in the control, — and the ascorbic acid content of the serum was 0.56 mg per 100 ml.

The ash content of the femur calculated on a dry basis was 56.8 % in the rat injected by whale liver oil, and 52,7 % in the rat injected by peanut oil.

In none of these experiments cramps occurred as a result of subcutaneous injection of large quantities of vitamin A oils, when great care was taken not to introduce the oil into the blood stream. It appeared feasible therefore, that the cramps reported by some previous workers, might be due to fat emboli as a result of the oil accidentally being introduced into blood vessels.

For the purpose of verifying this, one of the blood vessels on the thigh in a normal rat was carefully punctured by a thin needle, and two drops of a vitamin A oil ("Avipan A. L.") containing 20,000 I. U./g was injected. Shortly after the injection attacks of cramps occurred in the hind legs, and in the tail, as well as twitching around the nose and mouth of the rat. Later on the hind legs appeared paralyzed.

Table 12.

Manifestation of Symptoms in Rats Given Excess of Vitamin A by Subcutaneous Injection.

Experiment no.	Conditions of experiments	Rat no.	Initial body weight, g	Duration of experiment, days	Lethal result	Reduced weight gain	Poor condition	Alopecia	Soreness of skin	Exophtalmus	Swelling of palpebrae	Limping	Diarrhea	Proteinuria	Hyperemia	Subcutaneous hemorrhages
9.	50,000—130,000 I.U. vit. A daily, (bear and whale liver oil)	1	70	5	+	+	+	+	0	0	0	0	0	+	+	+
		2	90	34	0	+	+	+	+	+	+	+	0	—	+	0
10.	10,000 I. U. vit. A daily (vit. A emulsion)	1	100	33	0	+	+	0	+	0	+	0	0	—	—	0
		2	103	33	0	+	+	+	+	0	+	0	+	—	+	+
11.	1 mill. I.U. vit. A in a single injection	1	216	11	0	0	0	0	0	0	0	0	0	—	0	0
	Injection of peanut oil	1	162	7	0	0	0	0	0	0	0	0	0	—	0	0

Summary of Results from Experiments 9—11. From these experiments it may be concluded that hypervitaminosis A may also develop as a result of injection of bear or whale liver oil. The doses required to produce toxic symptoms are much larger, however, when the crude oil is injected subcutaneously, than the minimum dose producing toxic symptoms when given orally. This was probably caused by the fact that only small amounts of the oil were absorbed by subcutaneous injection. Alopecia was found not only at the place of injection, but also all over the body.

At the places of application, abscesses with pus secretion were found, which seems to indicate a lowered resistance against infection by hypervitaminotic rats, as no abscesses were found in normal rats which for other purposes were injected with oil free of vitamin A.

Symptoms of hypervitaminosis A were more readily produced when vitamin A was injected in an emulsive form. In this case 80—100 I. U. vit. A/g body weight daily caused distinct symptoms of hypervitaminosis A.

No ill effect followed a single subcutaneous injection of 1 million I. U. vit. A in the form of a highly concentrated whale liver oil, which was probably due to incomplete absorption of the oil. Subcutaneous injection

of the same quantity of peanut oil for control purposes was also harmless.

In none of these experiments cramps occurred as a result of subcutaneous injection of large quantities of vitamin A oils, when great care was taken not to introduce the oil into the blood stream, while attacks of cramps occurred shortly after the injection of small amounts of a vitamin A oil containing only 20,000 I. U. vit. A/g into the blood vessels.

5. *Hypervitaminosis A and Alopecia.*

Experiment 12.

The purpose of this experiment was to investigate the effect of vitamin A concentrate when applied locally on the skin of healthy rats. It has been observed that rats receiving large doses of vitamin A oils suffered from loss of hair around the mouth and nose, and also elsewhere on the body, and the question arose as to whether this alopecia was due to a local action of the oil, or whether it was an expression of the state of hypervitaminosis A.

The two rats used in this experiment had initial body weights of 50 and 100 grams, and the pelts looked perfectly normal at the commencement of the experiment. The rats were isolated in individual cages, and every day two drops of the bear liver oil corresponding to approximately 8,000 I. U. vit. A were smeared on the skin between the shoulder blades.

On the thirteenth day of the experiment the smaller of the two rats which had appeared weak and unwell shortly after the beginning of the experiment (apparently for some other reason than the bear liver oil) died. By examination, no loss of hair was found on the place where the oil had been applied, although the hair was covered with oil.

The treatment continued with the second rat until twenty-one days after the beginning of the experiment, when it was killed. No loss of hair was observed on the place of application or elsewhere.

The weight increase in the first rat was less than normal, but in the second rat it was normal.

From this experiment, it may be concluded that bear liver oil, as such, does not cause any loss of hair in normal rats when applied locally in amounts of two drops daily, corresponding to approximately 8,000 I. U. vitamin A.

6. *The Effect of Single Massive Doses of Vitamin A.*

For the purpose of examining the effect of large single doses of vitamin A given orally during a short period of time, the following two experiments were carried out.

Experiment 13.

Two male rats, with initial body weights of 70 and 239 grams respectively, were given large doses of whale liver oil concentrate (200,000 I. U. vit. A/g) at 30 minute intervals, by catheter, during a period of 3 hours. Simultaneously a dose

of 50,000 I. U. vit. A was mixed in the basal diet in order to obtain the highest possible intake of vitamin A.

The smaller of the two rats was given 700,000 I. U. vit. A in the course of 3 hours, and the larger rat 1.6 million I. U. vit. A during the same period.

As an immediate result of this treatment, weakness and scruffiness was observed within a few hours. The smaller of the two animals showed increasing weakness, and shivering and twitching occurred.

Following these large doses of vitamin A the rats were kept over night on the ordinary basal diet and were killed and examined 24 hours after the first dosage with vitamin A.

Both rats had lost considerable weight during this 24 hours period, — one of them as much as 5.5 grams.

Apart from a scruffy pelt, no symptoms were observed. They were lively, but suffered from diarrhea.

By postmortem examination, large amounts of unabsorbed fat were observed in the stomach and intestines. Apart from hyperemia, and swollen visceral lymph glands, no pathological findings were detected by postmortem macroscopical examination. Histologically, no significant pathological findings, except hyperemia, were detected in the kidney, adrenal, pancreas, testis or spleen. By sudan III staining of the liver, slight deposits of sudanophil droplets were seen scattered throughout the organ, both in and between the liver cells. The blood coagulated normally and the hemoglobin was 93 and 110 % respectively. Lipemia was observed in the adult rat but not in the younger one, — and the vitamin A content of the serum was 31.0 and 4.5 I. U. vit. A/g serum respectively.

Experiment 14.

In a second experiment, similar large doses of vitamin A were given to a normal adult male rat in the course of 2½ hours. It was then kept on an ordinary adequate basal diet and observed for a period of 11 days for the purpose of examining whether any delayed effect could be detected.

Following a total dose of 1.4 million I. U. vit. A given in the course of 2½ hours, the rat looked quiet, apatic and drowsy. Otherwise no immediate effect was observed.

During the first three days a marked loss of weight was observed (17 grams), followed by a weight increase which by the 10th day had gained up to the initial weight.

Oil was excreted through the anus even 4 days after the dose. At the end of 5 days, diarrhea was observed, which lasted throughout the experiment. At the end of 9 days, soreness of the eyes occurred, as well as swelling of the forehead, and the following day there was swelling of the palpebrae on one of the eyes.

By postmortem examination no pathological findings were revealed apart from the already described eye symptoms. The hemoglobin was 99 %, and the ascorbic acid content of the serum was 0.49 mg per 100 ml. The ash content of the femurs was 55.2 % calculated on a dry basis, and the calcium and phosphorus contents of the ash were 32.1 % and 17.1 % respectively.

Summary of Results from Experiments 13 and 14. From experiment 13 it may be concluded that oral administration of massive single doses of vitamin A in the form of whale liver oil concentrate — up to 1.6 million I. U. has no immediate lethal effect on rats, nor did a very high vitamin A level in the blood (31 I. U. vit. A/g serum) in itself prove lethal.

From experiment 14 it is further evident that single massive doses of vitamin A, apart from symptoms of acute intoxication shortly after the dose, and loss of weight, caused only a slight delayed effect on rats.

7. *The Toxic Dose of Vitamin A.*

Experiment 15.

As a preliminary investigation into the approximate gross dose of vitamin A sufficient to cause symptoms of hypervitaminosis A in young growing rats, a group of four rats with initial body weights between 67 and 83 grams (average 74.0) was given 10,000 I. U. vit. A daily by dropping pipet in the form of whale liver oil concentrate. The average gross dose of vitamin A per gram body weight was 135 I. U. at the beginning of the experiment, and 47 I. U. at the conclusion of the experiment.

A second group of four rats with similar initial body weights (72.0 —89.0, average 80.3 g) was given 50,000 I. U. vit. A daily in the form of the same whale liver oil by dropping pipet. The average gross dose of vitamin A at the beginning of the experiment was 625 I. U./g body weight, and towards the end of the experiment it was 188 I. U./g body weight.

Apart from the different doses of vitamin A in the two groups, the condition of the experiment was identical. They all received the usual adequate basal diet in unlimited quantities.

In the urine collected from the first group receiving 10,000 I. U. vit. A daily from the 24th day of the experiment, the Heller's test, as well as the acetic acid test were faintly positive, and by microscopical examination of the centrifuged urine, a few red blood cells were seen per field of vision. Schlesinger's test was negative. The secreted amount of urine was less than 1 ml per rat, and the excreted amount of ascorbic acid was 0.0 mg/rat in 24 hours.

The benzidine test in the feces was negative at the beginning of the experiment, and positive towards the end of the experiment after 10—15 seconds. The feces contained 11.8 % fat.

During the experiment which lasted from 53—58 days, no clinical symptoms were observed apart from slightly reduced weight gain. One of the rats was killed and examined at the end of 53 days, and the remaining four at the end of 58 days. By macroscopical examination, no pathological findings were made. Three of the rats were females, and they were all pregnant. In all cases the blood coagulated normally. The results of various laboratory examinations are given in table 15. From this table it will be seen that in all cases the hemoglobin was normal. The ascorbic acid content of the serum varied between 0.43 and 0.83 mg/100 ml, and the ascorbic acid content of the liver varied between 12 and 18 mg per 100 g. Serum colour was normal.

Microscopical examination of the liver, kidneys, adrenals, pancreas and intestines, from three of the rats in this group revealed slight or moderate hyperemia, a few red blood cells in the spaces of Bowman's capsules in the kidney and slight degeneration of some of the convoluted tubules. By sudan III staining of the liver, only a very few sudanophil droplets were seen scattered throughout the whole organ in all cases, mostly situated between the liver cells (Kupffer's cells).

In the adrenals a well defined narrow strip of abnormally dense sudanophil deposits were seen in the zona glomerulosa, while only very few sudanophil droplets were seen in the rest of the cortex, except in one case where the entire cortex was packed with dense sudanophil deposits. No sudanophil droplets were seen in the medulla. In the rest of the organs, no pathological findings were revealed.

X-ray examination of the long bones at different stages of the experiment showed normal conditions in all cases.

In the second group, receiving 50,000 I. U. vit. A daily, the following observations were made:

The weight gain was reduced, and towards the conclusion of the experiment a marked loss of weight was observed.

The feces contained approximately 12 % fat, as against 10 % in the control group receiving basal diet only. The benzidine test in the feces gave varied results, although it was distinctly positive on the 15th day after 10 seconds.

A urine sample was collected on the 20th day, at which time Heller's reaction was negative, while microscopical examination of the centrifuged urine showed many red blood cells per field of vision. The excreted amount of urinet was 1 ml per rat in 24 hours.

Two of the rats were males, and two females, and although they were kept together in the same cage throughout the experiment, which lasted altogether 86 days, neither of the females became pregnant during this period.

Distinct symptoms of hypervitaminosis A were observed in all rats at the end of 6—14 days. Limping occurred in two rats at the end of 6 days, in one at the end of 9, and in one at the end of 30 days. Fractures occurred in one rat after 20 days, in two rats after 30 days, and in one at the end of 66 days. All rats appeared scruffy and where in very poor condition at the end of 19 days. Soreness around the eyes and mouth was observed in all rats. In one rat alopecia occurred at the conclusion of the experiment. Swelling of the palpebrae and exophtalmus was observed in two rats. In one rat, blood was observed on the tail without it being possible to decide whether it had been a matter of urogenital or intestinal hemorrhage. In all cases healing of the fractures occurred with marked callus formation and the healing appeared complete within one month after the fracture. Most of the fractures were located at the humerus. In one case limping of the hind legs occurred at irregular intervals, without it being possible to detect any fracture by X-ray examination, or any hemorrhage by postmortem examination.

X-ray examination of the long bones at various stages of the experiment revealed changes typical for hypervitaminosis A.

The hemoglobin was determined in blood collected from the tail of three of the rats at the end of two months, and again at the conclusion of the experiment, at the end of 74 and 84 days. The results are tabulated as follows:

Table 13.

Hemoglobin in Hypervitaminotic Rats at Different Stages of the Experiment.

Rat no.	Sample collected at the end of 60 days	Sample collected at the end of 74 days	Sample collected at the end of 84 days
1	89	74	-
2	102	-	died
3	98	-	76

From table 13 it appears that while the hemoglobin showed normal values at the end of two months of the experiment it was considerably reduced 14—24 days later. In one of these cases (rat no. 1) this may be due to hemorrhages around the fracture, which occurred 6 days after the first blood sample had been taken. In the other case the reduced hemoglobin content towards the conclusion of the experiment can hardly be due to hemorrhages associated with fractures, since fractures in this case had occurred long before the first sample had been collected. Furthermore, in all of these cases only slight hemorrhages were detected around the fractures by postmortem examination. It thus appears likely that the anemia cannot entirely depend on macroscopical hemorrhages.

By postmortem examination only slight hemorrhages were detected around the fractures. Apart from this no subcutaneous, visceral or muscular hemorrhages were detected by macroscopical postmortem examination, except in one of the rats which suddenly died at the end of 78 days, and in which hemorrhage was detected in the left adrenal at autopsy, and the kidney was embedded in a large hematoma approximately 4 cm long (see ill. 34). By histological examination the hemorrhage appeared to have originated from the adrenal which showed large necrotic areas. The were dense deposits of pigment between the kidneys and along the aorta. There were a large number of swollen lymph glands between the kidneys, along the aorta, in the thymus, lungs and on both sides of the thyroid glands. These were particularly numerous in the lungs. Apart from these there were some organs between the kidneys which resembled accessory adrenals. The liver was fatty on the cut surface.

In the remaining rats there was marked fragility of the bones in all cases. The livers were light in colour and appeared fatty on the cut surface. Dense deposits of pigment were seen in two of the rats between the kidneys, along the aorta, around the thymus gland, and along the sternum and ribs. Accessory adrenals and swollen visceral lymph glands were observed in all cases. In one of the rats the thymus gland and the spleen appeared enlarged.

Histological examination of the liver, kidneys, adrenals, pancreas, intestines, lungs, the thyroid gland and bones in two of the rats revealed marked hyperemia in the lungs, kidney and liver. In the previously described rat which suddenly died at the end of 78 days, there were distinct signs of pneumonia in the lungs with alveoli filled with exudate containing red blood cells, leucocytes and macrophages, and several focal abscesses in the lung tissue, containing neutrophil granulocytes. In the kidney a large amount of pigment was seen throughout the organ. There was degeneration and necrosis of certain areas, both involving the glomeruli and tubules, some of which were filled with necrotic cells and deposits of calcium. A large number of vacuoles were seen in the liver, and the Kupffer cells appeared swollen. In the bones (tibia) there was hyperemia, the periostal blood vessels being dilated and packed with red blood cells, and subperiostal hemorrhages. There were signs of increased osteoclastic activity, and irregularity of bone structure in the metaphysis. By sudan III staining of the liver, very dense deposits of particularly large sudanophil droplets were seen throughout the organ, both in and between the liver cells, particularly in the Kupffer cells. In the adrenal, a narrow band with abnormally dense sudanophil deposits was seen in the zona glomerulosa of the cortex. In one case slight fatty degeneration was observed in the renal tubules.

The results of various laboratory examinations are given in table 15.

Summary of Results from Experiment 15. From this experiment it may be concluded that 135—47 I. U. vit. A per gram body weight

(10,000 I. U. vit. A daily) caused only slight toxic symptoms with slight reduction in the weight gain and slight hematuria and proteinuria as well as positive benzidine reaction in the feces, while 50,000 I. U. daily or 625—188 I. U./g body weight, caused distinct symptoms of hypervitaminosis A. In the first case the ascorbic acid content of the blood and the liver was practically normal or slightly reduced. The hemoglobin was normal in all cases. No pathological changes were observed in the bones, and no pathological findings were revealed by macroscopical postmortem examination, while histological examination revealed findings similar in type to those observed in the group given 50,000 I. U. vitamin A daily, but less pronounced.

In the second case, when 50,000 I. U. vit. A was given daily, distinct clinical symptoms occurred at the end of 6—14 days, with considerably reduced weight gain, limping, fractures, changes in the pelt such as scruffiness, soreness and alopecia, — poor general condition and eye symptoms. Pronounced changes were detected in the bones. There was hematuria, and the benzidine test in the feces was positive. Slight anemia was observed in two cases, and reduced ascorbic acid content of the serum in the one case examined, as well as increased serum colour, and prolonged prothrombin time. Postmortem examination revealed pronounced pathological findings.

No significant abnormalities were detected in the ash and mineral contents of the bones in any of the groups (see table 16).

While all female rats in the first group receiving 10,000 I. U. vit. A daily became pregnant during the experiment, pregnancy occurred in none of the females receiving 50,000 I. U. vit. A daily.

8. *The Effect of Excess of Vitamin A in Rats at Various Stages of Development.*

In our previous experiments it appeared that the tendency to spontaneous fractures in hypervitaminotic rats was less in older animals than in young growing rats. Adults rats appeared generally less affected by the same gross dose of vitamin A than very young rats. It was therefore considered desirable to examine the effect of excess of vitamin A in rats at various stages of development, from very young to adult animals, when kept under identical conditions.

a) **The Effect of Excess of Vitamin A in Very Young Rats.**

Experiment 16.

For the purpose of studying the effect of excess of vitamin A in very young rats, a group of 5 animals with initial body weight between 22 and 43 grams were given 50,000 I. U. vit. A daily in the form of whale liver oil concentrate, by dropping pipet.

Table 14.

Results of Various Laboratory Examinations in Rats Given Different Doses of Vitamin A. (Experiment 15.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Lethal result	Reduced weight gain	Weakness	Scruffiness	Alopecia	Soreness of skin	Swelling of palpebrae	Exophthalmus	Limping	Fracture	Pathological X-ray findings	Proteinuria	Hematuria	Benzidine test in feces	Pneumonia	Subcutaneous hemorrhages	Visceral hemorrhages	Muscular hemorrhages	Fatty cut surface of liver	Swollen visceral lymph glands	Hyperemia	Red blood cells outside capillaries	Red blood cells in space of Bowman's capsule	Sudanophil deposits in liver	Increased sudanophil deposits in adrenal cortex	Degeneration of renal tubules
Basal diet + 10,000 I. U. vit. A daily (135—147 I. U. vit. A/g body weight).	1	♂	67	58	0	+	0	0	0	0	0	0	0	0	0	+		+	0	0	0	0	0	0	-	(+)	-	+	(+)
	2	♂	83	58	0	+	0	0	0	0	0	0	0	0	0				0	0	0	0	0	0	-	(+)	+	+	-	-
	3	♀	77	58	0	+	0	0	0	0	0	0	0	0	0				0	0	0	0	0	0	+	-	+	+	-	-
	4	♀	69	53	0	+	0	0	0	0	0	0	0	0	0				0	0	0	0	0	0	+	-	+	+	-	-
Basal diet + 50,000 I. U. vit. A daily (625—1188 I. U. vit. A/g body weight).	1	♂	72	86	0	+	+	+	0	+	+	+	+	0	+			+	0	0	0	0	+	+	+	0	-	+	-	-
	2	♂	73	78	+	+	+	+	0	+	0	0	+	+	+				0	(+)	(+)	(+)	+	+	+	+	+	+	-	-
	3	♂	87	84	0	+	+	+	0	+	0	0	+	+	+				0	(+)	(+)	(+)	+	+	+	+	+	+	-	-
	4	♀	89	40	0	+	+	+	+	+	+	+	+	+	+				0	0	0	0	+	+	+	+	+	+	+	+

Table 15.

Results of Various Laboratory Examinations in Rats Given Different Doses of Vitamin A. (Experiment 15.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, million per 1/1000 ml	Colour index	mg ascorbic acid in 100 ml serum	Serum colour	Fasting blood sugar mg/100 ml	Prothrombin time, seconds	P in serum, mg/100 ml	mg ascorbic acid in 100 g liver
Basal diet + 10,000 I. U. vit. A /day	1	♀	67	58	100	-	-	0.56	-	-	-	-	18
	2	♂♀	83	58	104	-	-	0.78	-	-	-	-	18
	3	♀	77	58	102	-	-	0.43	-	-	-	-	12
	4	♀	69	53	91	-	-	0.83	3	-	-	-	-
	Mean:		-	-	99	-	-	0.65	-	-	-	-	16
Basal diet + 50,000 I. U. vit. A /day	1	♀	72	86	74	4.6	0.8	-	-	-	-	-	-
	2	♂♀	73	78	-	-	-	-	-	-	-	-	-
	3	♂	87	84	76	4.5	0.8	0.13	10	-	25	5.1	-
	4	♀	89	40	88	5.4	0.8	-	-	90	-	-	-
	Mean:		-	-	79	4.8	0.8	-	-	-	-	-	-

Table 16.

Ash and Mineral Contents of the Femurs in Rats Given Different Doses of Vitamin A. (Experiment 15.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % in dry bone	Ca, % in ash	P, % in ash	Length of femur, mm	Width of femur, mm	Fractures
Basal diet + 10,000 I. U. vit. A daily	1	♀	67	58	57.8	32.4	17.3	32	2.5	0
	2	♂	83	58	61.2	34.7	17.4	40	2.5	0
	3	♀	77	58	58.2	34.2	18.2	30	2.5	0
	4	♀	69	53	59.3	32.9	16.6	30	2.4	0
	Mean:		-	-	59.1	33.6	17.6	33	2.5	-
Basal diet + 50,000 I. U. vit. A daily	1	♀	71	86	50.0	38.8	21.9	29	2.0	+
	2	♂♀	73	78	57.6	37.1	19.3	33	2.0	+ +
	3	♂	87	84	61.0	37.4	18.0	33	2.3	+ +
	4	♀	89	40	54.8	32.9	17.4	31	2.0	0
	Mean:		-	-	55.9	36.6	19.1	32	2.1	-

Table 17.

Ash and Mineral Contents of the Femurs in Very Young Hypervitaminotic Rats. (Experiment 16.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Basal diet + 50,000 I.U. vit. A daily (1300—830 I.U. vit. A/g body weight)	1	♀	43	18	41.3	36.4	25.6	21	1.8	+
	2	♂	38	18	44.6	31.5	22.7	21	1.3	+
	3	♀	42	18	32.2	35.5	21.9	-	-	+
	5	♀	42	18	34.9	34.3	22.6	-	-	+
	Mean:		-	-	38.3	34.3	23.2	21	1.6	-

All rats in this group were from the same litter, and were 27 days old at the commencement of the experiment. They were given the ordinary adequate basal diet in unlimited quantities. The average daily dose of vitamin A during the experiment corresponded to between 1,300 and 830 I. U. vit. A/g body weight.

Distinct symptoms of hypervitaminosis A were detected in all rats in this group within 10 days. Following an increase in the vitamin A dose from that day, a distinct loss of weight was observed as well as rapidly increasing symptoms. All rats suffered from marked weakness, alopecia, scruffiness, as well as multiple fractures. The general condition in these rats was particularly poor (see ill. I).

Towards the conclusion of the experiment, 0.0 mg ascorbic acid was excreted through the urine per rat in 24 hours. One of the rats suffered from marked hematuria.

One of the rats died at the end of 13 days, and three at the end of 18 days. The remaining rat was killed and examined when moribund on the 18th day. The fating blood sugar was 60 mg per 100 ml in this case, as against 88 in the control. The hemoglobin was 83 %, red blood cells 4.2 millions per 1/1000 ml, the colour index being 1.0. At autopsy the bones were found to be very brittle, and broke easily by grasping with an ordinary forceps. The adrenals were dark in colour, and accessory adrenals were seen along the aorta. The liver was fatty on the cut surface. There were scattered hemorrhages in the meninges.

Postmortem examination of the other rats in this group revealed similar findings: hyperemia, emaciation, scattered small subcutaneous hemorrhages, clubbing of the costochondral junction, bone fragility, accessory adrenals along the aorta and fatty appearance of the cut surface of the liver. In no case was there any signs of infection or pneumonia, but in one of the rats there was marked cyanosis just prior to death.

By microscopical examination of three of these rats, marked hyperemia was observed in all cases in the internal organs, such as the liver, kidneys, and ovaries. The Kupffer cells in the liver appeared swollen. In the kidney there was slight degeneration of some of the convoluted tubules. Free blood was seen in some of the collecting tubules, and in the space of Bowman's capsule in some places. Apart from hyperemia, no pathological findings were detected in the ovaries. By sudan III staining, massive deposits of sudanophil droplets of varying size were seen in the liver, — both in and between the liver cells, and throughout the adrenal cortex. No sudanophil deposits were seen in the kidney.

X-ray examination of the long bones at various stages of the experiment revealed, apart from the fracures, abnormally thin bone shafts, thinning of the cortical shadow, which was absent at the proximal ends of the tibiae, mottled appearance of the epiphyses, — and lines of dense calcification at the epiphyseal border of the metaphyses.

From table 17 it appears that while the ash content of the bones appears to be less than the normal for rats of this age, the calcium and phosphorous contents of the ash are not reduced. It must be noted, however, that the pronounced changes present in the bones — in the form of subperiostal hemorrhages, fractures and irregularity of the structure of the bones, might well be responsible for the difference in the ash content.

b) **The Effect of Excess of Vitamin A in Adolescent and Adult Rats.**

Experiment 17.

The original purpose with this experiment was to examine the effect of excess of vitamin A on the skeletal system in adolescent rats, — with initial body weights between 80 and 140 grams. As this experiment extended over a period of several months, however, the findings also refer to the conditions in adult animals. At the same time the opportunity was taken to study the fertility in hypervitaminotic rats, as well as the young from the hypervitaminotic mothers. For practical purposes this will be considered in a later chapter in connection with the description of similar repeated experiments in other groups of rats (see page 73). In the present connection we shall only deal with the manifestation of hypervitaminosis A in adolescent and adult rats, and a comparison of the symptoms in male and female animals.

In this experiment, a group of six rats with initial body weights between 80 and 140 grams were, in addition to the usual adequate basal diet, given whale liver oil concentrate corresponding to an average of approximately 50,000 I. U. vit. A daily by dropping pipet, or 500—250 I. U. vit. A/g body weight. The potency of the oil was checked at intervals and found to remain unchanged throughout the experiment, when the oil was stored in refrigerator temperature. All rats in this group, three of which were male and three female, were kept together in the same cage.

On the third day of the experiment, all rats in this group looked very scruffy, and were in a poor condition, and at the end of 16 days a distinct fracture was detected by X-ray examination in one of them. In the course of the experiment, fractures occurred in all three male rats, but only in one of the female rats. In this rat pregnancy did not occur, while the remaining two female rats produced young, — one of them twice during the experiment. The pregnant rats appeared less affected by the excess of vitamin A than the other rats in this group.

Urine was collected, in the previously described manner, from the whole group at various stages of the experiment, during twenty-four hour periods, for the examination of the presence of protein, blood and sugar.

Altogether 10 urine samples were collected during the period from the 10th to the 62nd day of the experiment, and in all cases the Heller's test and the acetic acid test were positive. The Esbach test showed 1 ‰ protein at the end of 24 hours. Haine's test for sugar gave negative results. Microscopical examination of the centrifuged urine revealed no red blood cells in the first sample, while

many red blood cells per field of vision were constantly detected in all samples examined after the 36th day of the experiment.

The benzidine test in the feces was negative during the first part of the experiment. On the 50th day it was positive after ten seconds, and on the 62nd day after five seconds.

Clinical symptoms and postmortem findings will be described for each rat in the following:

Rat No. 1 (male): Following the initial symptoms already mentioned, swelling (oedema) and soreness of the eye were observed on the 8th day. Two days later both eyes were affected, the rat was quiet and appeared very weak. The eye symptoms disappeared after a few days, but reappeared on the 16th day. Eye symptoms of varying degree came and went at irregular intervals throughout the experiment. At times the eyes were practically closed from swelling of the palpebrae. Towards the conclusion of the experiment, however, the eye symptoms persisted constantly. On the 34th day a limping gait was observed, which became more pronounced in the following couple of days. It then disappeared, but occurred again distinctly on the 48th day, at which time exophtalmus was also observed. From the 50th day the general condition of the rat was particularly poor. It looked very miserable, the pelt being in a very poor condition. Four days later, alopecia was also observed, as well as limping of the right fore leg, and X-ray examination revealed fracture of the right humerus. At the conclusion of the experiment, X-ray examination revealed also fractures of the left tibia and fibula.

The rat was killed and examined in the usual manner at the end of 65 days. The results of blood examinations are given in table 18.

By postmortem examination large blood crusts were observed around the right eye. Large subcutaneous hemorrhages were found around the fracture of the left fore leg, but apart from this no subcutaneous or visceral hemorrhages were observed. Otherwise no significant pathological findings were made.

Microscopical examination revealed marked hyperemia in the internal organs and the bones. Scattered red blood cells were seen outside the capillaries and in the space of Bowman's capsule in the kidney. Apart from hyperemia no significant pathological changes could be detected in the testes. The epididymis, which appeared normal, was filled with sperms. The pancreas, spleen and salivatory glands were normal. By sudan III staining of the liver, dense deposits of sudanophil droplets were seen throughout the organ, both in and between the liver cells, particularly in the Kupffer cells, which appeared swollen. In the adrenal, considerable deposits of sudanophil droplets were seen unevenly distributed throughout the cortex. In the tibia, great irregularity was observed of the bone structure. There were signs of increased osteoclastic activity and fracture of compact bone towards the metaphyses, where it was very irregular and thin.

X-ray examination of the long bones at various stages of the experiment, revealed, apart from the already mentioned fractures, an increasing thinning of the bone shafts, the centre of the shafts being mostly affected, as well as abnormally thin cortex, which had completely disappeared at the proximal ends of the tibia. Deformities in the cortex at the upper end of the tibia were also observed, as well as lines with abnormally dense calcification at the epiphyseal borders. Bone changes were observed at the end of one month of the experiment, and became more pronounced in the course of the experiment.

Rat No. 2 (male): Signs of acute intoxication and initial symptoms, as previously described. On the 6th day the left eye was swollen and running, and two days later both eyes were affected. In this rat the eye symptoms were also

inconsistent, occurring at irregular intervals. On the 10th day the rat was quiet and looked very weak.

On the 13th day of the experiment there was marked swelling of both eyes, as well as pronounced muscular weakness. On the 16th day, fracture of the left fore leg was clinically diagnosed. Twenty-two days after the beginning of the experiment there was a limping of the left hind leg, indicating a possible fracture. X-ray examination 6 days later, however, confirmed a distinct fracture of the left fore leg, but no fracture of the hind leg. Later X-ray examination revealed healing of the fracture in spite of the fact that the vitamin A had been given continuously in unchanged doses.

At the end of four weeks after the beginning of the experiment, limping was also observed on the other hind leg. At the end of 41 days, the eyes were practically closed from swelling of the palpebrae. Exophtalmus was observed on the 48th day, at which time the rat was in particularly poor condition. This condition persisted for several days. Eye symptoms were again present approximately two monts after the beginning of the experiment, at which time X-ray examination revealed fractures of both hind legs in addition to the previously mentioned fracture of the left fore leg.

The rat was killed and examined in the usual manner 65 days after the beginning of the experiment. The results of blood examinations are given in table 18.

X-ray examination of the long bones revealed, apart from the fractures, similar changes as described for the previous rat (ill. 14).

By postmortem examination, hemorrhages were observed around the fractures. The liver was very light grey in colour and appeared fatty on the cut surface. The entire colon was taken up by a hard tumour which had a diameter of about 15 mm, and which had an uneven surface, the intestinal lumen being very narrow. By microscopical examination it was found to be an adenoma.

Rat No. 3 (male): Following the initial symptoms as described for the previous rats in this group, swelling of the palpebrae occurred on the 8th day of the experiment. Two days later, dyspnoea was observed. The rat was very quiet and looked weak.

The swelling of the eyes persisted for several days, until the 20th day after the beginning of the experiment. It then disappeared, but reoccurred on the 41st day, when both eyes were practically closed by the marked swelling of the palpebrae.

At the end of 19 days, the pelt was very thin, as a result of marked alopecia. Limping as well as fracture of one toe occurred on the 44th day. Four days later, fracture of the left fore leg was clinically diagnosed, at which time the general condition of the rat was particularly poor. The fracture was verified by X-ray examination. Fifty-seven days after the beginning of the experiment, there was again a marked swelling of the palpebrae, which persisted until the rat was killed.

The rat was killed and examined 69 days after the beginning of the experiment. The results of blood examinations are given in table 18.

X-ray examination of the long bones revealed similar findings as described for the previous rats in this group.

By postmortem examination, the rat was found to be in a good condition. The blood coagulated normally. The liver was large, the weight being 12.7 g and was pale in colour. The adrenals were very dark in colour. Several accessory adrenals were detected, as well as swollen visceral lymph glands around the kidneys and on both sides of the thymus gland. Apart from this no pathological findings were made.

By microscopical examination, marked hyperemia was observed in the internal organs. A few red blood cells were seen in the space of Bowman's capsule in

the kidney in some places. There was also slight degeneration of some of the renal tubules, some of which were filled with amorphous masses, and deposits of calcium. A large number of vacuoles were seen in the liver. No significant pathological findings were detected in the spleen.

Rat No. 4 (female): The same initial symptoms as described for the previous rat occurred on the 3rd day, at which time limping was also observed, as well as marked swelling of the palpebrae. This limping disappeared in the course of a few days, but the eye symptoms persisted in varying degrees until the 10th day, at which time the rat was very quiet and weak. The eye symptoms occurred irregularly at long intervals. Apart from this, no other symtoms were observed until the 48th day, when limping occurred, which persisted during two days, at the end of which time the general condition was poor. The limping disappeared but reoccurred a week later, being very indistinct. X-ray examination of the long bones at the end of two months, revealed negative results, except for a slight thinning of the bones shafts.

On the 65th day the rat produced seven young, at which time there was slight limping and eye symptoms with slight loss of hair around the eyes. From the 73rd day this rat was kept in a separate cage together with a normal adult male rat, which was given ordinary basal diet. The female rat was given the same dose of vitamin A continuously, and in addition to this 50 mg ascorbic acid daily by subcutaneous injection, during a period of 46 days. During this period, the rat appeared perfectly normal, except for a faint swelling of the palpebrae on the 83rd day. A distinct improvement was observed in the general condition of the animal during this treatment, particularly in the pelt, which became quite normal.

X-ray examination of the long bones at the conclusion of the experiment, revealed no fractures, but a slight thinning of the bone shaft as already described.

The rat was killed and examined on the 119th day of the experiment.

The results of the blood examinations are given in table 18. By postmortem examination, no symptoms were detected by exterior examination. The adrenals were enlarged. The liver was very light in colour and appeared to contain a larger number of lobes than normal, some of which were very small.

Microscopical examination revealed degeneration and necrosis of renal tubules, some of which were filled with red blood cells (see ill. 31). Free blood was also seen in the space of Bowman's capsule in several places. Large deposits of sudanophil droplets were seen in the liver, both in and between the liver cells (in the Kupffer cells which appeared swollen) when stained by sudan III. In the adrenal, dense sudanophil deposits were seen in the zona glomerulosa, while only moderate or slight deposits were seen in the rest of the cortex. There was a distinct widening of the costochondral junction which had a conical form. The zone of calcification appeared broadened. The compact bone was very thin and irregular. In the teeth, there was hyperemia in the pulp, and deposits of calcium. There was great irregularity of the odontoblasts, some of which appeared degenerated.

Rat No. 5 (female): During the first two months of the experiment, the female rats were kept together with adult male hypervitaminotic rats, and while pregnancy occurred in the other two female rats, it did not occur in this rat.

Following the initial symptoms previously described, swelling of the palpebrae (oedema) occurred on the 8th day of the experiment. This swelling persisted several days and was distinctly noticeable the 10th day of the experiment, at which time the rat was very quiet and appeared to be weak. Marked swelling of the palpebrae was again observed on the 42nd day, when the eyes were practically closed. Two days later, there was a slight limping of the left hind leg. On the

48th day exophtalmus was observed, and the general condition of the rat was poor. These symptoms persisted, and on the 74th day X-ray examination revealed a distinct fracture of the right hind leg, which was rapidly healing in spite of excess of vitamin A being given in unchanged doses. The limping persisted nevertheless (ill. 16).

The rat was killed and examined in the usual manner at the end of 119 days after the beginning of the experiment. X-ray examinations at intervals throughout the experiment revealed similar changes as described for the male rats.

The results of blood examinations are given in table 18.

By postmortem examination no pathological findings were detected by exterior examination. Swollen lymph glands were observed around the left kidney and around the thymus. The were slight visceral hemorrhages.

Microscopical examination of the liver, kidney, adrenals and costochondral junction revealed identical findings to those described for rat no. 4. In the teeth, hyperemia and a large number of vacuoles were seen in the pulp, as well as irregular arrangement of odontoblasts. In the tibia there was hyperemia and great irregularity of bone structure.

Rat No. 6 (female): Following the previously described initial symptoms, weakness was observed on the 10th day, and eye symptoms on the 13th day. Slight alopecia was observed on the 20th day, and marked swelling of the palpebrae was observed on the 40th day, both eyes being practically closed. A few days later the rat appeared quite normal and at the end of 53 days it produced 7 young, two of which were dead when born. One month after the first birth, the rat again produced 6 young.

Apart from slight eye symptoms from the 56th—58th day, the rat appeared normal until it was killed the 119th day, in spite of the vitamin A being given in unchanged doses.

The rat was killed and examined in the usual manner. The results of blood examinations are given in table 18.

X-ray examination of the long bones showed practically normal conditions apart from slight thinning of the bone shafts.

By postmortem examination the liver was fatty on the cut surface and weighed 8 grams. The adrenals were considerably enlarged and there was markedly increased pigmentation around them, aong the aorta, around the thymus gland, and on both sides of the sternum. Swollen lymph glands were seen around the thymus gland. Otherwise no pathological findings were made.

By microscopical examination the following findings were made:

Kidney: Moderate hyperemia, slight degeneration of some of the renal tubules, some of which were filled with amorphous masses, and a calcium-like substance. There were no deposits of sudanophil droplets in this kidney stained by sudan III.

Adrenal: A large number of vacuoles throughout the cortex. When stained by sudan III a very dense sudanophil zone was seen in the zona glomerulosa, just inside the capsule. Dense deposits of sudanophil droplets were also seen in the rest of the cortex. A few sudanophil droplets were also seen in the medulla.

Liver: Hyperemia and a large number of vacuoles. When stained by sudan III very dense deposits of large sudanophil droplets were seen throughout the organ, particularly between the liver cells.

Pancreas: Hyperemia.

Table 18.

Results of Blood Examinations in Adult Hypervitaminotic Rats. (Experiment 17.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	Hemorrhages	mg ascorbic acid per 100 ml serum	Prothrombin time, seconds	serum colour	Total bases in serum, milliequiv/l	Phosphorus, mg/100 ml	I.U. vit. A/g serum, 24 hours after last dose
Basal diet + excess of vit. A, 50,000 I.U. daily (500—250 I.U. vit. A/g body weight)	1	♂	90	65	78	5.5	0.7	+	0.74	-	-	-	-	-
	2	♂	81	65	69	5.4	0.6	+	0.85	-	-	-	-	-
	3	♂	139	69	87	5.2	0.9	0	0.59	-		-	-	-
	4	♀	102	119	86	5.3	0.8	0	0.07	22.5	10	149.7	-	-
	5	♀	83	119	74	4.6	0.8	(0)	0 13	26.5	12	-	5.4	-
	6	♀	114	119	88	5.2	0.9	0	-	-	-	-	-	2.8
	Mean:		-	-	80	5.2	0.8	-	0.48	24.5	11	-	-	-
Basal diet (control)	1	♂	49	95	106	5.2	1.0	0	0.65	-	-	-	-	-

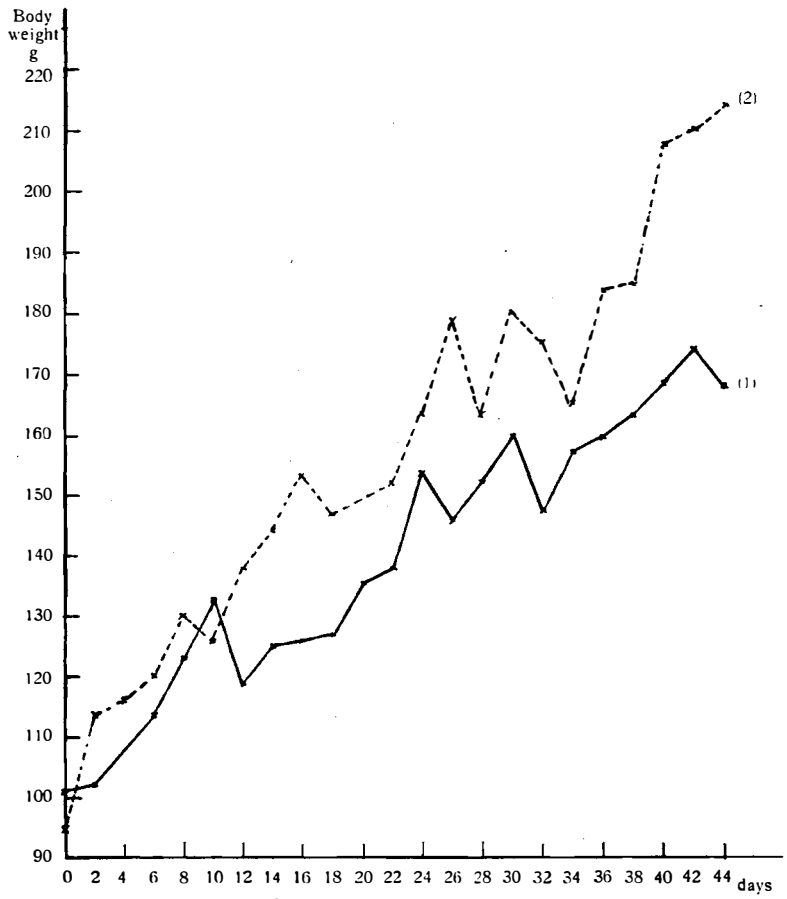
Table 19.

Ash and Mineral Contents of Bones in Adult Hypervitaminotic Rats. (Experiment 17.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Basal diet + excess of vit. A 50,000 I. U. daily, (500—250 I.U. vit. A/g body weight)	1	♂	90	65	53.3	35 4	17.4	34	2.0	+
	2	♂	81	65	49.2	45.9	19.5	-	-	++
	3	♂	139	69	55.7	35.4	19 1	35	2.1	+
	4	♀	102	119	55.0	32.4	18.3	30	2.2	0
	5	♀	83	119	51.2	32.1	17.5	30	1.9	+
	6	♀	114	119	57.1	28.4	17.1	31	2.1	0
	Mean:		-	-	53.6	34.9	18.2	32	2.1	-
Basal diet (control)	1	♂	49	95	50.1	39.4	18.3	-	-	0

From table 18 it is evident that in this experiment the hemoglobin is lower in the hypervitaminotic rats than in the control, but only in 3 out of the 6 rats was there a distinct hypochromic anemia, in two of which, large hemorrhages were detected, which might explain the anemia. In the third case only slight visceral hemorrhages were observed.

From table 19 it is evident that no abnormalities were detected in the ash and mineral contents of the bones.



Graph No. 5. Average weight graphs for adult rats given 250—500 I.U. vit. A/g body weight daily (1), compared with normal rats (2). (Experiment 17.)

c) **The Effect of Excess of Vitamin A in Adult Rats.**

Experiment 18.

In one experiment, six adult rats with initial body weights between 156 and 176 grams were given 25,000 I. U. vit. A daily in the form of whale liver oil concentrate mixed in the ordinary basal diet, corresponding to less than 150 I. U. vit. A/g body weight.

A second group of six animals with approximately the same initial body weight was used as a control, and was given the basal diet only.

From the average weight graph on page 67 a marked difference in the weight increase in the two groups is evident.

One of the rats in the first mentioned group died at the end of 18 days, with marked symptoms of hypervitaminosis A. By postmortem examination no macroscopical pathological findings were made, except for a very enlarged adrenal (weight of left adrenal was 0.0582 g corresponding to 0.035 % of the body weight), which was fiery red and had a fatty appearance of the cut surface. The vitamin C content of the adrenals was 60 mg/100 g.

The remaining rats in this group were killed at the end of one month, at which time they had all developed symptoms of hypervitaminosis A. By post-mortem examination, no pathological findings were made, except for enlarged adrenals, and fatty cut surface of the liver.

In two of these rats, the liver and adrenals, and in one of them also the kidneys and testes were examined histologically. Hyperemia was observed in all these organs. There was slight degeneration of some of the renal tubules, some of which were filled with necrotic masses, and free blood in the space of Bowman's capsule. In the liver, stained by sudan III, massive deposits of sudanophil droplets were observed throughout the organ, particularly between the liver cells. In the adrenal, very dense deposits of sudanophil droplets were seen in the cortex, particularly in the zona glomerulosa. A few scattered sudanophil droplets were also seen in the medulla.

Twenty-four hours before being killed, a blood sample was collected from the tail in one of these rats for the determination of the prothrombin time, which was 14.1 seconds (normal). All the rats in this group, including this rat, were then given 10 mg vitamin K (2-methyl-1: 4-naphtoquinone). They were killed 24 hours after this, and blood samples were collected. In the first mentioned rat the prothrombin time was unchanged (14.3 seconds), and in all cases it was normal (14.1—15.0 seconds).

The results of other blood examinations are given in table 20. From this table it is observed that the hemoglobin was distinctly reduced in two cases. The ascorbic acid content in the serum was less than normal in two out of four cases.

The control rats remained normal throughout the experiment.

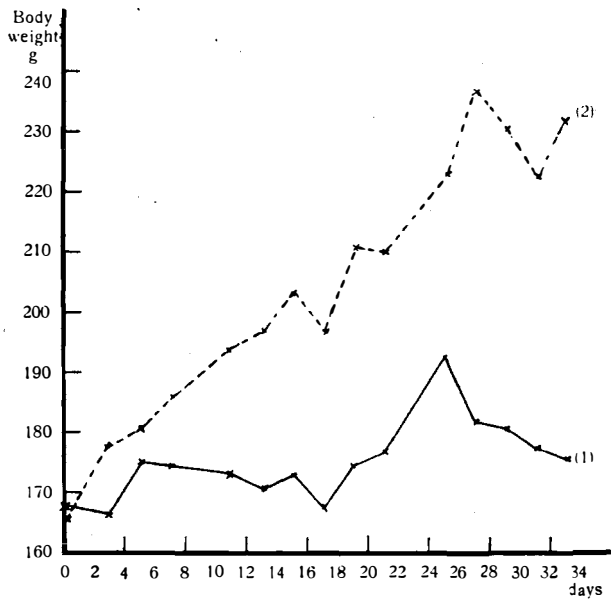
Table 20.

Results of Various Laboratory Examinations in Adult Rats Given 25,000 I. U. Vitamin A Daily, Mixed in Basal Diet. (Experiment 18.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Weight of left adrenal, g	Weight of left adrenal in % of body weight	Ascorbic acid in adrenals, mg/100 g	Prothrombin time, seconds	Prothrombin time after oral administration of vit. K, seconds	Hb %	mg ascorbic acid in 100 ml serum
Basal diet + 25,000 I. U. vit. A daily (< 150 I.U. vit. A/g body weight)	1	♀	160	18	0.0582	0.035	60	-	-	-	-
	2	♂♀	174	34	-	-	-	-	15.0	93	0.29
	3	♂♀	170	34	-	-	-	-	14.2	56	0.46
	4	♂	176	34	-	-	-	-	-	104	0.71
	5	♀	166	34	-	-	-	-	14.6	84	0.29
	6	♀	156	34	-	-	-	14.1	14.3	67	-
	Mean:		-	-	-	-	-	-	14.5	81	0.44

Experiment 19.

In a further experiment a group of six adult rats with initial body weights between 150 and 246 grams (average 234 grams), were given 50,000 I. U. vitamin A daily in the form of whale liver oil concentrate



Graph No. 6. Average weight graphs for adult rats given 25,000 I.U. vit. A daily (<150 I. U. vit. A/g body weight) mixed in the basal diet (1), compared with normal rats (2).

mixed in the ordinary sufficient basal diet. The dose corresponded to approximately 200 I. U. vit. A/g body weight.

Two of the rats were killed at the end of 38 days, two at the end of 52 days, and two at the end of 55 days.

In three of the rats, loss of weight was observed. In the remaining three the weight was practically the same at the end of the experiment as the initial weights, although a slight weight fall had been observed some time after the commencement of the experiment. Eye symptoms with swelling of the palpebrae, exophtalmus and soreness around the eyes were observed in all rats in this group, as well as an increasing weakness. Fractures were observed in two of the rats, and a marked bending of the spine was observed in three rats.

By postmortem examination the adrenals appeared greatly enlarged, and were very dark in colour in two cases. The liver was light in colour and appeared fatty on the cut surface.

Histologically, marked hyperemia was observed in the internal organs in the two rats examined. In the liver, stained by sudan III, massive deposits of sudanophil droplets were seen throughout the organs, both in and between the liver cells, particularly in the Kupffer cells, which were swollen (ill. 30). In the adrenal, only moderate deposits of sudanophil droplets were seen in the cortex, except for a well-defined narrow strip in the zona glomerulosa, where the deposits were dense.

In one case the prothrombin time was prolonged (22 seconds), while in a second case it was normal (16 seconds). The potassium content of the serum was 4.5—5.7 milliequivalents/liter, as against 4.9 milliequivalents in the control. The average vitamin A content of the visceral fat was 660 I. U. vit. A/g. In two cases the vitamin C content of the serum was examined after 24 hours fasting, and was found to be 0.43 and 0.45 mg per 100 ml.

The hemoglobin was 74 % in two cases, as against 81 %, 93 % and 102 % in the control.

Table 21.

Results of Various Laboratory Examinations in Experiment 19.

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	mg ascorbic acid in 100 ml serum,	Prothrombin time, seconds	K in serum, milliequiv./l	I.U. vit. A/g visceral fat.	Hb %
Basal diet + 50,000 I.U. vit. A daily (200 I.U. vit. A/g body weight)	1	♀	210	38	0.43	-	-	660	-
	2	♀	152	38	0.45	-	4.5		-
	3	♀	213	51	-	-	5.7		74
	4	♂	246	52	-	-	5.7		74
	5	♀	238	55	-	22	-		-
	6	♂	210	55	-	16	-		-
	Mean:		-	-	0.44	19	5.3	660	74

Experiment 20.

A final group of six adult rats with initial body weights between 143 and 192 grams (average 163 g), were given 100,000 I. U. vit. A daily in the form of whale liver oil concentrate in addition to the basal diet (625 I. U./g body weight). Half of the dose was given mixed in the diet, and the other half by catheter.

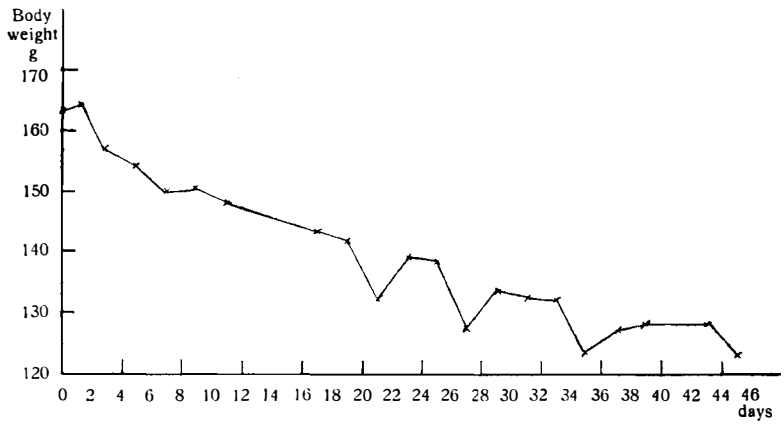
Two of the rats were males, and the other four were females. One of the rats was moribund at the end of 28 days, and the surviving rats were killed and examined at the end of 46 days, at which time a second rat was moribund.

A marked loss of weight was observed in all rats in this group (see average weight graph on page 69).

Urine was collected in specially constructed cages for examination during 24 hour periods, on the 25th, 34th, 42nd and 45th day of the experiment. Simultaneously urine samples were collected from control rats for comparison. The excreted amount of urine per rat during 24 hours varied between 1.3 and 5.0 ml per rat in the hypervitaminotic animals, the average being 2.5 ml as aginst 5—6 ml in the normal rats. In all cases, the urine from the hypervitaminotic rats was much darker in colour than the urine from the control rats.

Since the antimony-trichloride test in repeated samples gave negative results in the urine, it was concluded that no vitamin A was excreted through the urine in hypervitaminotic rats. 0.0 mg ascorbic acid was excreted in the urine per rat during 24 hours. Haine's test for sugar was negative. In all cases the Heller's test, the acetic acid test and the benzidine test was positive, — and these tests became more strongly positive towards the conclusion of the experiment. The Esbach's test showed from ½‰—1 ‰ after 24 hours. In all cases microscopical examination of the centrifuged urine showed many red blood cells per field of vision, and the number of red blood cells increased towards the conclusion of the experiment.

In the urine from the normal control rats, the Heller's test, the acetic acid test, the benzidine test, as well as the Esbach's test showed negative results, and by microscopical examination no red blood cells were detected in the centrifuged urine.



Graph No. 7. Average weight graph for six adult rats given 100,000 I.U. vit. A daily (625—765 I.U. vit. A/g body weight).

In the first urine sample, which was collected in the usual metabolism cage, the urine was contaminated by feces. In order to examine whether this feces contamination had any effect on the Heller's test, the acetic acid test, and the benzidine test, fresh feces from the same group was extracted thoroughly by water. In the filtered extract, the Heller's test as well as the acetic acid test were negative, while the benzidine test was positive after 20 seconds. The benzidine test in the feces was positive after 10 seconds. From these observations it was concluded that the observed proteinuria was certain, and that the tests had not been influenced by the feces contamination, while it could not be decided whether the positive benzidine reaction, and the presence of red blood cells in the centrifuged urine might be the result of the urine being contaminated by feces.

In other experiments on the other hand, the benzidine test, as well as microscopical examination of the urine had given distinctly positive results when contaminated by feces, while the benzidine test in the feces gave negative results. Furthermore, in some cases, urine contaminated by feces gave negative benzidine tests, while the same test in the same feces gave positive results. Finally in several cases, macroscopical hematuria was observed in urine collected directly from hypervitaminotic rats. It must therefore be concluded that hematuria in some cases is a definite symptom in hypervitaminotic rats.

The remaining urine samples were collected in a specially constructed cage, which allowed automatic separation of the urine and feces, and prevented contamination of the urine, and the results were as previously mentioned. The benzidine reaction in the feces from the hypervitaminotic rats in this experiment was in all cases, except one, strongly positive after 2—5 seconds.

In this experiment, the male rats appeared less affected by the overdosage with vitamin A than the female rats, as judged by clinical examination. In both male rats there was a marked weakness, apart from the distinct loss of weight, and in one of them marked swelling of the palpebrae occurred on the 25th day.

Of the female rats, one was killed and examined when moribund on the 28th day, the remaining being killed and examined at the same time as the male rats, — on the 46th day of the experiment. All female rats had multiple fractures, — two of them having fractures of both fore legs, and two of them fractures of all four extremities. The fractures occurred between the 19th and the 28th day of the experiment. Apart from these fractures, there were also eye symptoms

ranging from slight swelling of the palpebrae to marked oedema which closed both eyes, and soreness around the eyes and nose, as well as on the tail. In one case there was bleeding from the nose. In addition to the above mentioned fractures, the hind legs appeared paralysed in one case (see ill. 5), without any fracture or hemorrhage being detected in the hind legs. Diarrhea was observed in one case, and one of the rats suffered from marked dyspnoea. The pelt looked scruffy in all cases, and in one case there was marked alopecia around the anus. A sharp bending of the spine was observed in all rats in this group.

The rats were killed and examined in the usual manner. The results of various laboratory examinations are given in table 22.

In all cases the blood coagulated within 5 minutes as judged by the rough clinical method, described on page 21.

By postmortem examination enlargement of the adrenals, fatty appearance of the cut surface of the liver, which was abnormally light in colour, as well as hyperemia was observed in all cases. In one case the lungs were fiery red without any signs of pneumonia being detected. This rat, which was moribund on the 46th day of the experiment, was very restless and suffered from dyspnoea, and suddenly died before blood samples could be collected. Apart from hemorrhages around the fractures, no subcutaneous or visceral hemorrhages were detected by macroscopical postmortem examination. In one of the rats with multiple fractures, there were no signs of healing of the fractures, and no visable hemorrhages.

The hind legs appeared to be paralysed in the rat which died on the 28th day of the experiment, and by postmortem examination no fractures or hemorrhages were detected which might be taken as the cause of this. There were fractures of the fore legs, however, with considerable muscular hemorrhages around the fractures. In this case the hemoglobin was 70 %. There was also marked hyperemia, but apart from this no pathologico-anatomical findings were revealed.

Four of the rats were examined histologically, and in all cases hyperemia was observed in the internal organs and the bones. In the kidney, slight degeneration of the renal tubules was detected in one rat, and scattered red blood cells in the space of Bowman's capsule in another rat. Apart from hyperemia, no significant pathological findings were made in the pancreas and testis. By sudan III staining of the liver massive deposits of large sudanophil droplets were seen throughout the organ, particularly between the liver cells, in all cases. In the adrenal, abnormally dense deposits of sudanophil droplets were observed in the zona glomerulosa and in one case the entire cortex was packed with sudanophil deposits (see ill. 43). A few scattered sudanophil droplets were detected in the renal tubules, some in the intestinal mucosa, and considerable sudanophil deposits in the lungs, but none in the heart and spleen. In the tibia great irregularity was observed in the bone structure in the metaphysis, with scarcity of bone spicules.

The average vitamin C content of the serum was less than normal (0.29 mg/100 ml, as against 0.83 in control rats), as was also the vitamin C content of the liver (see table 22). The urea content of the blood was approximately the same as in normal control animals (49.4 mg/100 ml, as against 43.4—52.5 in control rats). The weight of the adrenal was higher than normal. There was no reduction in the ash and mineral content of the bones (see table 23).

From this experiment it is evident that 100,000 I. U. vit. A, i. e. approximately 625—765 I. U. vit. A/g body weight, produced pronounced symptoms of hypervitaminosis A in adult rats, with loss of weight, general malady and fractures, but apart from bleeding around the fractures, no hemorrhages were observed. It is thus evident that hemorrhages are not more frequent in adult rats than in young animals, and that fractures occur equally as frequently in adult rats as in young rats when given massive doses of vitamin A.

Table 22.

Results of Laboratory Examinations in Adult Rats Given 100,000 I. U. Vitamin A Daily. (Experiment 20.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ascorbic acid in serum, mg/100 ml	Urea in serum, mg/100 ml	Hb %	Weight of left adrenal, g	Weight of left adrenal in % of body weight	Ascorbic acid in liver, mg/100 g
Basal diet + 100,000 I.U. vit. A daily (625—765 I.U. vit. A/g body weight)	1	♂	163	46	-	-	-	0.0337	0.027	12
	2	♂	192	46	-	-	-	0.0319	0.020	16
	3	♀	149	28	-	49.4	70	0.0267	0.027	10
	4	♀	170	46	0.13	-	-	0.0347	0.029	8
	5	♀	161	46	-	-	-	0.0307	0.031	6
	6	♀	143	46	0.45	-	-	0.0250	0.021	18
	Mean:		-	-	0.29	-	-	0.0304	0.026	12

Table 23.

Ash and Mineral Contents of the Femurs in Adult Rats Given 100,000 I. U. Vitamin A Daily. (Experiment 20.)

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Basal diet + 100,000 I.U. daily (625—765 I.U. vit. A/g body weight)	1	♂	163	46	59.5	45.6	18.3	30	2.2	0
	2	♂	192	46	59.5	30.7	17.9	31	2.0	0
	3	♀	149	28	59.1	32.4	17.2	29	2.3	+
	4	♀	170	46	61.6	33.0	17.8	32	2.2	+
	5	♀	161	46	60.0	28.3	17.6	30	2.1	+
	6	♀	143	46	56.9	31.1	18.4	30	2.2	+
	Mean:		-	-	59.5	33.5	17.9	30	2.2	-

Summary of Results from Experiments 16, 17, 18, 19, and 20. From these experiments it may be concluded that the clinical picture as well as the postmortem findings are similar in both young, adolescent and adult rats, and that they are equally affected when similar gross doses of vitamin A per gram body weight are given. Contrary to the findings previously reported (Moore and Wang, 1945; Walker, Eylenburg and Moore, 1947), there were in these experiments no higher incidence of

Table 24.

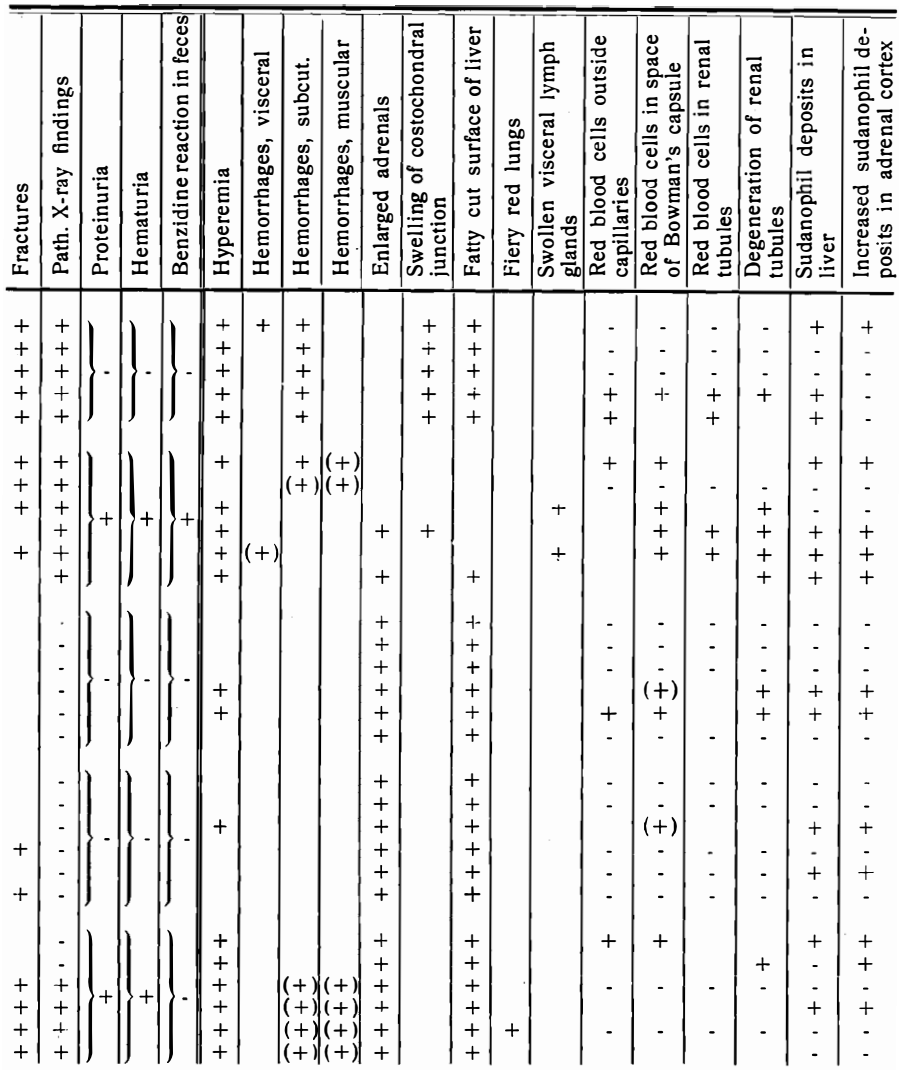
Manifestation of Clinical Symptoms, and Postmortem Findings in Rats

Experiment no.	Conditions of experiments	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Lethal result	Reduced weight gain	Weakness	Lack of activity	Scruffiness	Alopecia	Soreness of skin	Swelling of palpebrae	Exophthalmus	Diarrhea	Limping
16.	Basal diet + 50,000 I.U. vit. A daily (1300—830 I U. vit. A/g body weight)	1	♀	43	18	+	+	+		+	+					+
		2	♂	38	18	+	+	+		+	+					+
		3	♀	42	18	+	+	+		+	+					+
		4	♂	21	13	+	+	+		+	+					+
		5	♀	42	18	+	+	+		+	+					+
17.	Basal diet + 50,000 I.U. vit. A daily (500—250 I.U. vit. A/g body weight)	1	♂	90	65	0	+	+	+	+	+	+	+	+		+
		3	♂	81	65	0	+	+	+	+			+	+		+
		3	♂	139	69	0	+	+	+	+	+		+			+
		4	♀	102	119	0	+	+	+	+	+		+			+
		5	♀	83	119	0	+	+	+	+			+	+		+
		6	♀	114	119	0	+	+	+	+	+		+			
18.	Basal diet + 25,000 I.U. vit. A daily (< 150 I.U. vit. A/g body weight)	1	♀	160	18	+	+	+	+	+		+	+			
		2	♀	174	34	0	+							+		
		3	♂	170	34	0	+					+		+		
		4	♂	176	34	0	+									
		5	♀	166	34	0	+							+		
		6	♀	156	34	0	+							+		
19.	Basal diet + 50,000 I.U. vit. A daily (200 I.U. vit. A/g body weight	1	♀	210	38	0	+	+				+	+	+		
		2	♀	152	38	0	+	+				+	+	+		
		3	♂	213	52	0	+	+			+	+	+	+		
		4	♂	246	52	0	+	+				+	+	+		-
		5	♀	238	55	0	+	+				+	+	+		
		6	♂	210	55	0	+	+				+	+	+		-
20.	Basal diet + 100,000 I.U. vit. A daily (625—765 I.U. vit. A/g body weight)	1	♂	163	46	0	+	+		+			+			
		2	♂	192	46	0	+	+		+						
		3	♀	149	28	+	+			+		+	+			+
		4	♀	170	46	0	+			+		+	+			+
		5	♀	161	46	+	+	+		+	+	+	+		+	+
		6	♀	143	46	0	+			+		+	+			+

hemorrhages (subcutaneous or visceral) in adult rats than in young rats, and the same bone changes occurred, although these appeared to develop more slowly in adult rats than in young growing animals.

While the ash content of the femurs was not reduced in adolescent and adult hypervitaminotic rats, it was less than normal in very young hypervitaminotic rats. The calcium and phosphorus contents of the ash, however, were not reduced in any of these groups.

of Different Age, Given Excess of Vitamin A.



9. Fertility in Hypervitaminotic Rats, and the Young from Hypervitaminotic Mothers.

The uterine hemorrhages in pregnant rats given excess of cod-liver oil reported by Hartwell (1927) were reproduced by Moore and Wang (1945) also in animals given halibut liver oil, or vitamin A acetate. They interpreted this hemorrhage as a special manifestation of the general liability to hemorrhage in hypervitaminosis A. In their experiments they found that even when the young were born alive, they were not reared

unless the mother's ration was changed to a mixed diet of natural foodstuffs.

In connection with the present investigations the fertility in hypervitaminotic rats was examined, as well as the young from hypervitaminotic mothers.

Three female rats with initial body weights between 80 and 114 grams were kept together with three male rats with initial body weights between 90 and 140 grams during a period of several months (see experiment 17, page 59). All rats received 50,000 I. U. vitamin A daily (500—250 I. U./g body weight) in the form of whale liver oil concentrate, by dropping pipet in addition to the usual adequate basal diet.

At the end of two months, one of the female rats was diagnosed pregnant. A week later it gave birth to seven young. As the excess of vitamin A had been given during a considerable time to both the male and female rats, — and as all rats at the time of conception showed signs of hypervitaminosis A, these young must be considered the offspring of hypervitaminotic parents. It is thus evident that 500—250 I. U. vit. A did not affect conception or pregnancy in this case.

By examination immediately after birth, the young appeared normal. They were of normal size and weight, — and appeared healthy.

The following day, however, one day after birth, — four of the young were dead. Apart from cyanosis, no pathological findings were made by postmortem examination. There were no signs of violence. Microscopical examination revealed marked hyperemia in the liver, heart, lungs, kidneys, adrenals, pancreas, bones and in the pulp of the teeth. There was thickening of the alveolar walls in the lungs, and slight degeneration of the renal tubules. Scattered red blood cells were seen outside the capillaries in the periost of the tibia as well as in the muscles. Otherwise no pathological findings were made.

The following day, the mother had eaten two of the young, — and the remaining baby died the next day.

The mother was now, for the purpose of examining whether the result would be different when the rat was mated with a normal male, kept together for a period of a month with a normal male rat, which was given the usual adequate basal diet only. The femle rat received 50,000 I. U. vitamin A daily by dropping pipet continuously in addition to the basal diet. Pregnancy, however, did not occur.

In the second rat, pregnancy failed to develop when kept together with hypervitaminotic males during a period of approximately two months. The rat was then kept together with a normal male during a period of one month, — with the same negative result.

The third of the female rats produced seven young at the end of 53 days from the beginning of the experiment, two of which were probably born dead, and one of them was eaten by the mother immediately after birth.

By postmortem examination of the dead babies, no pathological findings were detected. By microscopical examination, similar findings as mentioned above were made, such as hyperemia in the internal organs and bones. Scattered red blood cells were seen outside the capillaries in the muscles and connective tissue of the lower extremities, and in the liver tissue.

Two of the living babies were killed and examined immediately after birth. By postmortem examination normal organs were found. The hemoglobin was 51 % and 73 %.

Four days later, the remaining two babies were moribund. There was no sign of violence. Just prior to death, cramps were observed, and death occurred suddenly. Microscopical examination revealed hyperemia in the kidney, liver, pancreas and bones. A large number of vacuoles were seen in the liver, and a few red blood cells in some of the spaces of Bowman's capsules in the kidney. The lungs showed normal findings. When stained by sudan III, a considerable number of small sudanophil droplets were seen in and between the liver cells throughout the organ. In the adrenal the entire cortex was packed with sudanophil droplets. In the tibia there were signs of increased osteoclastic activity with large Howship's lacunae filled with cells. In one place subperiostal hemorrhages were seen.

The rat was again mated with a hypervitaminotic rat, and a second litter of 6 babies was born one month after the first birth. All were born alive, and showed normal size and weight at birth, but the skin appeared very dry. One of the babies appeared very weak, and the following day it died. At this time the remaining five babies appeared normal, except for the dryness of the skin. The average weight of the young when one day old, was 4.6 g as against 4.8 g in the control. The length of the young (without the tail) was 4.2 cm, as against 4.5 cm in the control. They all suckled, — their stomachs being filled with milk.

In the following days, lactation took place normally, although the young failed to gain weight. At the end of 14 days, one of them was much smaller than the others. It was very weak and appeared moribund. It was completely free of hair, and appeared slightly yellow (jaundice?). At this time the hair had begun to grow out in the other young, but there was denudation in patches. All the hypervitaminotic babies appeared drowsy.

The following day, the smaller of the babies was eaten by the mother, and the remaining babies were small and their general condition was very poor.

When they were 18 days old, the average weight of the young was 13.6 grams, the average daily weight increase being 0.5 grams during the first 18 days, as against 1.0 grams in the control. The average length (excluding the tail) was 6.9 cm. At this time one of the young had a fracture of the left hind leg, and the general condition of this baby was particularly poor.

The following day, another one was eaten by the mother, after having appeared weak and scruffy, with a yellowish colour of the skin the previous day.

When 20 days old, the young appeared distinctly abnormal. They were smaller than the young from the control group of the same age, and they showed decreased activity. The body weight was between 10 and 16 g, as against 25 g for normal young of the same age. The length was 6.5—8 cm, as against 8.5—9 cm for the normal.

The young received ample quantities of milk from the mother, and there was no evidence of the fact that they did not get enough food. Simultaneously it was observed that the mother was free of symptoms, in spite of the excess of vitamin A being given continuously in unchanged doses.

The following day the smallest of the three babies was eaten by the mother, and one of the remaining two was killed and examined in the usual manner. The length of the young (21 days old) was 6.5—7 cm, excluding the tail. The stomach was filled with milk, the hair was very light in colour, and the costochondral junctions appeared clubbed. The hemoglobin was 60 %, the red blood cells 3.36 millions per 1/1000 ml, colour index: 0.9.

Up to now the babies had only suckled from the mother. At this time (approximately 3 weeks after birth), the remaining baby discontinued the lactation, and began to take the ordinary basal diet, and ordinary cows milk. In the course of two days following this change of diet, a remarkable improvement in the general

condition of the baby was observed. Whereas before it had been very drowsy, it now appeared lively, and moved about in the cage and rapidly gained weight. The weight on the 23rd day (2 days after the lactation had been discontinued) was 22.5 grams. It appeared much stronger. X-ray examination of the long bones at this stage of development showed a characteristic roentgenogram (see ill. 19): thinning of the cortical shadow, a broad dense shadow around the centre of ossification, an irregular broadened intensely calcified zone at the epiphyseal end of the tibia, a glass transparency of the shaft with obliteration of the trabecular structure.

The baby was kept on the usual basal diet during a period of 20 days. A rapid weight increase followed the mentioned change in diet (see graph no. 8, p. 77), and at the same time, the general condition rapidly improved, except for the pelt, which looked scruffy throughout the experiment.

It was killed and examined in the usual manner at the age of 41 days, — 20 days after the mother's milk had been removed from the diet. During the latter period the average daily weight increase was 3.1 grams.

By postmortem examination it was found to suffer from diarrhea. Apart from a scruffy pelt, no symptoms were observed. There were no macroscopical hemorrhages. The liver was light in colour, and appeared fatty on the cut surface. The blood coagulated normally. There was swelling of the costochondral junctions. The heart appeared scarificated in a small area around the apex.

Histologically, considerable hyperemia was observed in the kidney, adrenal and liver (ill. 27). In the latter a large number of vacuoles were seen throughout the organ. In the kidney red blood cells were seen outside the capillaries in some places, and free blood in the space of Bowman's capsule, and in some of the renal tubules. There was also slight degeneration of some of the tubules. In the liver stained by sudan III considerable deposits of sudanophil droplets were seen in and between the liver cells. There was no fatty degeneration of the kidney.

The hemoglobin was 82 %, and the ascorbic acid content of the serum was 0.86 mg per 100 ml.

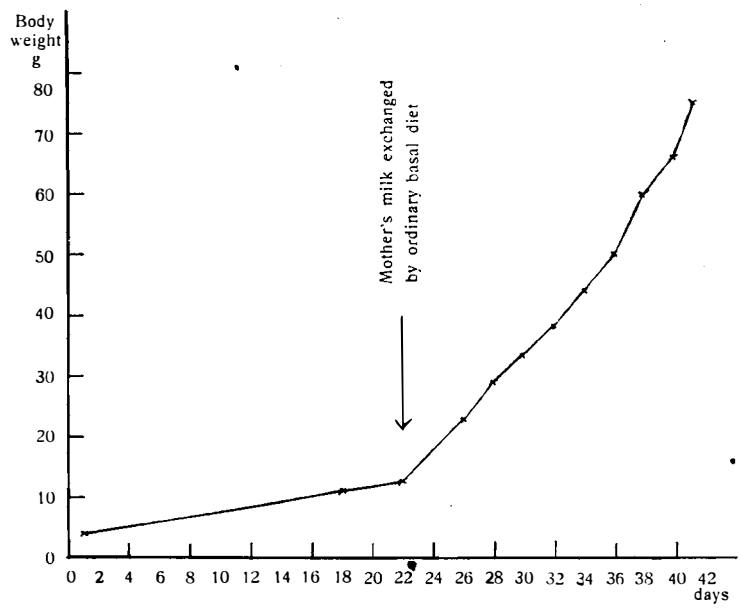
The ash content of the femur was 44.0 % calculated on a dry basis, and the calcium and phosphorus content of the ash was 34.7 and 22.8 % respectively.

In none of our experiments with hypervitaminotic rats, was there any evidence indicating that the babies were born too early, and no case of abortion was registered.

From these observations it appears unlikely that sufficient transplacental transfer of vitamin A takes place to produce a state of hypervitaminosis A in the foetus while intra-uterine. The young are born with normal size and weight, although weakness and skin abnormalities are observed in some cases, which might indicate that the foetus are slightly affected also in intra-uterine life.

In a further experiment (see experiment 20, page 68), 100,000 I. U. vitamin A daily (625 I. U. vit. A/g body weight) was given to a group of six adult rats, two of which were males and four females, during a period of 46 days. The vitamin A was given in the form of whale liver oil concentrate (200,000 I. U./g.) Half of the dose was given mixed in the basal diet, the other half by catheter. All rats in this group were kept together in the same cage throughout the experiment, without pregnancy occurring in any of the rats.

In another experiment, four adult female rats were kept together with two adult male rats in the same cage during a period of 38 to 55 days (see experiment 19, p. 66). The initial body weight in these rats was between 150 and 246 grams (average 234 grams). All rats in this group were given 50,000 I. U. vitamin A daily in the form of whale liver oil concentrate mixed in the ordinary adequate basal diet from the beginning of the experiment. This dose corresponded to an



Graph No. 8. Weight graph of a young rat from hypervitaminotic mother, showing rapid weight increase after the mother's milk was exchanged with ordinary basal diet.

average of approximately 200 I. U. vit. A per gram body weight. During the mentioned period all rats in this group developed distinct symptoms of hypervitaminosis A, and none of the rats became pregnant.

In a final experiment (experiment 18, see page 65), a group of six adult rats with initial body weights between 156 and 176 grams (average 167 grams) were given 25,000 I. U. vit. A daily in the form of whale liver oil concentrate mixed in the ordinary basal diet. This dose corresponded to less than 150 I. U. vit. A/g body weight.

Two of the rats were males, and four females, and they were kept together in the same cage for the purpose of mating during a period of one month.

During this period symptoms of hypervitaminosis A occurred in all rats, and only one of the rats became pregnant. In this case conception must have occurred before the commencement of the experiment as pregnancy was diagnosed already on the 10th day. On the 15th day symptoms of hypervitaminosis A were detected, the general condition rapidly became worse, and during partus on the 18th day of the experiment, the rat died without being able to give birth to the young. It looked anemic, and prior to death, short-lasting attacks of cramps in the limbs were observed.

By postmortem examination 9 foetus were observed. There were no abnormal hemorrhages and the foetus appeared normally developed, the length and weight being normal.

In the mother there were no subcutaneous or visceral hemorrhages, and no signs of infection. The liver was light in colour, and appeared fatty on the cut surface. The adrenals were enormously enlarged, and fiery red. The weight of the left adrenal was 0.0582 g, or 0.035 % of the body weight.

Otherwise, no pathological findings were revealed by macroscopical postmortem examination. Histological examination of the uterus, placenta, and foetus revealed no pathological findings.

The vitamin A content of the foetus (total) was 11 I. U. vit. A/g, as against approximately 2 I. U./g in foetus from the normal control rats. The average vitamin A content of the livers in the foetus from the hypervitaminotic mother was 150 I. U./g, as against 34 I. U./g in livers of foetus from normal mothers. The average vitamin A content of the placentas from the hypervitaminotic rat was 5.5 I. U. vit. A/g.

Table 25.

Vitamin A Content of Foetus from Hypervitaminotic Rat, Compared with Normal Foetus (Average).

	I.U. vit. A/g liver	I.U. vit. A/g placenta	I.U. vit. A/g foetus (total)
Foetus from hypervitaminotic mother	150	5.5	11
Normal foetus at same stage of development	34	-	2

From these observations it is evident that the vitamin A content of the livers in foetus from hypervitaminotic mothers is much higher (4—5 times higher) than in livers in foetus from normal mothers. This indicates a considerable transplacental transfer of vitamin A, although this transfer appears usually to be insufficient to produce hypervitaminosis A in the foetus during the intra-uterine life.

In the control group, consisting of the same number of animals, with the same initial body weights, and identical living conditions except for the excess of vitamin A, three out of four female rats became pregnant during the same period.

Summary of Results. From these observations it is evident that a higher incidence of sterility was detected in the hypervitaminotic rats, than in the normal animals. In one experiment pregnancy failed to occur when 50,000 I. U. vitamin A was given daily (200 I. U./g body weight), as was the case when 100,000 I. U. vitamin A was given daily in another experiment (625 I. U./g body weight). In a third experiment one rat became pregnant when given 25,000 I. U. vitamin A daily (150 I. U./g body weight), but in this case conception had taken place before the commencement of the experiment. In one group given 50,000 I. U. vitamin A daily (250—500 I. U./g) two out of three female rats produced young, one of them twice.

The incidence of pregnancy was not increased when the hypervitaminotic rats were mated with normal rats.

There was no evidence in any of our hypervitaminotic rats indicating that the babies were born too early, and no case of abortion was observed. Nor were any abnormal uterine hemorrhages detected in the pregnant rats.

In all cases the young appeared normal, except in one case when dryness of the skin was observed, and weakness which was observed

in all cases. The weight and size of the young when newly born was normal.

A high incidence of mortality was observed among the young, however, when fed by the hypervitaminotic mothers, as well as a marked reduction in growth. A rapid weight gain and improvement of the general condition in the young was observed when the mother's milk was removed and substituted by the ordinary basal diet.

The vitamin A content of the livers in foetus from hypervitaminotic mothers was 4—5 times higher than in livers in foetus from normal mothers indicating a considerable transplacental transfer of vitamin A.

10. *The Relation Between the Vitamin A Content of Blood and Internal Organs, and the Clinical Symptoms of Hypervitaminosis A.*

In connection with the present investigations the vitamin A level in the blood and the vitamin A contents of some of the internal organs at various stages during the development of the hypervitaminosis A symptoms, were studied.

a) Vitamin A Level in the Blood.

The relation between the vitamin A level in the blood and the manifestation of clinical symptoms of hypervitaminosis A was examined in some groups of rats, some of which are described in detail elsewhere in this paper. For practical purposes these observations will be summarized in this chapter.

1. Young Animals.

A group of 6 male rats, with an average initial body weight of 79.5 grams, were given 60,000 I. U. vit. A daily in the form of whale liver oil in addition to the basal diet. Half of this dose was mixed in the basal diet, and the other half was given daily by dropping pipet. The average dose of vitamin A/g body weight throughout the experiment — 18 days — was 730 I. U./g.

A second group of 6 male rats with an average initial body weight of 80.6 grams were given 10,000 I. U. vit. A daily in the form of the same whale liver oil as given to the previous group, by dropping pipet, in addition to the usual basal diet. This dose corresponded to an average of 95 I. U. vit. A/g body weight.

A third group of 5 rats described in detail in chapter 12, page 91, with an average initial body weight of 83.7 grams were given 50,000 I. U. vit. A daily in the form of 10 drops of whale liver oil concentrate, of which 6 drops per rat was mixed in the basal diet, and 4 drops given by dropping pipet daily. This dose corresponded to an average of 570 I. U. vit. A/g body weight daily. In addition to this 1 mg vit. B_1 was given daily for the purpose of another experiment.

Finally a fourth group of two rats with an average initial body weight of 76.7 grams was used for control purposes, and was given the basal diet only.

In the case when excess of vitamin A was mixed in the diet, the food mixture was prepared fresh daily. The oil was mixed in a minimum of the diet, and when it was consumed, ordinary basal diet was given in unlimited quantities. When the oil was given by dropping pipet, two drops were given at a time at intervals.

In the first group, the rats were killed at the first manifestation of distinct clinical symptoms, and the vitamin A content of the blood was examined. Simultaneously rats from the second group were killed and examined for comparison.

In the first group, receiving 60,000 I. U. vit. A daily, the average daily dose during the first 10 days of the experiment was 776 I. U./g body weight. During this period the average weight increase was 1.9 % of the initial weight, or 0.15 g per day, as against 61.3 % and 4.7 g respectively in the control group. During the rest of the experiment (8 days), the rats lost weight (0.9 g daily). During the entire experiment, there was an average daily loss of weight of 0.3 g daily, as against an average daily weight increase of 4.4 g for the control group (see table 26).

All rats in this group showed symptoms of acute intoxication, and looked very scruffy on the 2nd day. The general condition rapidly became worse, with increased weakness from the 7th day. Soreness around the eyes and diarrhea was observed in two cases. In 3 of the rats fractures occurred, one rat was moribund at the end of 13 days, and one died at the end of 18 days. Alopecia, particularly around the anus, was observed in 2 rats. The surviving rats were killed and examined in the usual manner when the symptoms became distinct.

The blood coagulated within five minutes. The results of vitamin A determinations in the blood are given in table 32.

Table 26.

Vitamin A Doses and Growth in Rats Given Excess of Vitamin A, Compared with the Control Rats.

Conditions of the experiment	I.U. vit. A/g body weight			Weight increase, g/day		
	0—10 days	10—18 days	0—18 days	0—10 days	10—18 days	0—18 days
Basal diet (control)	0.2	0.2	0.2	+ 4.7	+ 4.0	+ 4.4
Basal diet + 10,000 I.U. vit. A daily	106	84	96	+ 3.0	+ 3.5	+ 3.2
Basal diet + 50,000 I.U. vit A daily	592	554	576	+ 0.4	+ 0.9	+ 0.7
Basal diet + 60,000 I.U. vit. A daily	776	714	731	+ 0.2	- 0.9	- 0.3

By postmortem examination, emaciation was observed in all cases. In one case no other pathological findings were detected. In the rat which died with pronounced symptoms of hypervitaminosis A at the end of 18 days, the lungs were fiery red, without any signs of pneumonia being detected. In this case there was free blood in the knee joints, as well as scattered subcutaneous and visceral hemorrhages. In the remaining five rats, no hemorrhages were detected, except for considerable hematomas around the fractures in the three cases where fractures occurred. The livers appeared fatty on the cut surface, and in one case accèssory adrenals were observed.

Microscopical examination of three of the rats revealed hyperemia in the internal organs in all cases. A large number of vacuoles were seen in the liver, the Kupffer cells appeared swollen, and in one case small necrotic areas were seen. There was slight degeneration of the renal tubules, some of which contained a few red blood cells, and some amorphous masses and necrotic cells. In one case, red blood cells were seen outside the capillaries in the intestinal wall. Otherwise no pathological findings were made.

X-ray examination of the long bones, revealed similar findings as described for the other hypervitaminotic rats. The ash and mineral contents of the femurs are given in table 27.

In the second group, receiving 10,000 I. U. vitamin A daily, the average daily dose per gram body weight was 106 I. U./g during the first 10 days. During this period the average weight increase was 36.7 % of the initial weight, corresponding to 3.0 grams per day, as against 61.3 % and 4.7 g respectively for the control group. During the rest of the experiment — 8 days — the dose was 84 I. U./g body weight, and the weight increase was 3.5 g/day, as against 4.0 g in the control group. During the entire experiment — 18 days — the average daily dose was 95.7 I. U./g, and the weight increase was 71.2 % of the initial weight, or 3.2 g/day, — as against 102.7 % and 4.4 g/day respectively for the control group (see table 26). The average weight graph is given on page 87.

Apart from the slightly reduced weight gain mentioned, all rats remained apparently normal and free of any symptoms throughout the experiment, except for one rat, where swelling of the palpebrae and soreness around the eyes was observed.

The individual rats in this group were killed and examined about the same time as rats from the previous groups, for comparison.

By macroscopical examination no pathological findings were revealed. Microscopical examination of three of the rats revealed slight hyperemia in the kidney, bones, and the pulp of the teeth. A few vacuoles were seen in the liver, and slight degeneration of some of the renal tubules, as well as a few red blood cells in the space of Bowman's capsule in one rat. By sudan III staining of the liver, a few scattered sudanophil droplets were seen in and between the liver cells. In the adrenal, abnormally dense deposits of sudanophil droplets were seen in the cortex, particularly in the zona glomerulosa. In the teeth several vacuoles were seen in the pulp and the odontoblasts appeared irregularly arranged in some places. In the tibia irregularity of the bone structure was observed in one case; the compact bone was very thin, and had completely disappeared in one place near the metaphysis. The testes, epididymis, thymus, salivatory glands, pancreas and spleen appeared normal.

The hemoglobin was determined in two cases, and was found to be 93 and 112 %, as against 104 % in the control group.

X-ray examination of the long bones revealed no significant pathological findings. The ash and mineral contents of the femurs are given in table 27.

The results of vitamin A determinations in the blood are given in table 28.

In the third group, receiving 50,000 I. U. vit. A daily, a considerable reduction of the weight gain was observed. The relation between the vitamin A dose and the weight gain throughout the experiment is tabulated in table 26.

In all rats in this group, distinct symptoms of hypervitaminosis A were observed, such as fractures, scruffiness, weakness, muscular atrophy, and eye symptoms. A detailed description of these animals is given in chapter 12, p. 91.

Apart from hemorrhages around the fractures, enlargement of the adrenals, and fatty appearance of the cut surface of the liver, — no significant pathological findings were detected by macroscopical postmortem examination. Microscopical

Table 27.

Ash and Mineral Contents of Femurs in Rats Given Excess of Vitamin A, Compared with Normal Rats.

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Basal diet (control)	1	♂	85	32	52.4	-	-	-	-	0
	2	♂	68	32	53.6	31.8	17.9	30	-	0
	Mean:		-	-	53.0	-	-	-	-	-
Basal diet + 10,000 I.U. vit. A daily (96 I.U. vit. A/g body weight)	2	♂	87	19	49 1	29.6	19.3	-	-	0
	3	♂	96	19	53.2	30.0	17.6	27	2.1	0
	4	♂	78	15	46.8	33.0	19 6	24	2.0	0
	6	♂	85	8	45.2	37.9	19.0	-	-	0
	Mean:		-	-	48.8	32.6	18.9	26	2.1	-
Basal diet + 60,000 I.U. vit. A daily (730 I.U. vit. A/g body weight)	1	♂	69	9	48.8	28.0	18 8	21	2.0	0
	2	♂	95	18	51.2	29.8	19.4	26	2.2	0
	3	♂	99	13	52.2	27.7	21.3	24	2.0	0
	4	♂	80	14	52.0	33.7	17.5	-	-	+
	5	♂	68	12	50 1	31.0	20.7	22	1.9	+
	6	♂	67	8	46.2	34.7	22.0	21	2.0	+
	Mean:		-	-	50.1	30.8	20.0	23	2.0	-

examination of the liver, kidneys, adrenals, pancreas, intestines, testes, lungs, heart, bones and teeth in two of the rats revealed similar findings as previously described for hypervitaminotic rats such as: hyperemia in the internal organs and a few red blood cells in the space of Bowman's capsule in the kidney. A large number of vacuoles were seen in the liver in one case. When stained by sudan III, dense deposits of sudanophil droplets were seen between the liver cells (in the Kupffer cells), and dense sudanophil deposits in the zona glomerulosa of the adrenal cortex. In the tibia, muscular and periostal hemorrhages were seen around the fractures. There was great irregularity of bone structure in the metaphyses, and the compact bone was very thin. Otherwise no pathological findings were made.

One of the rats was killed and examined when moribund on the 14th day of the experiment, two rats on the 20th day, and the remaining two on the 32nd day, after the excess of vitamin A had been removed for a period of 14 days.

The results of the vitamin A determinations in the blood are given in table 28.

2. **Adult Animals.**

The vitamin A level in the blood was also determined in adult male hypervitaminotic rats in connection with experiment 8, described in detail on page 38. In this experiment a group of six rats with an average initial body weight of approximately 200 grams were given 50—90,000 I. U. vit. A daily (260—480 I. U./g body weight) in addition to the basal diet. These rats were killed and examined at the first manifestation of distinct clinical symptoms, as judged by the occurrence of fractures, in order to examine the level of the vitamin A in the blood, associated with the occurrence of manifest clinical symptoms.

Table 28.

Vitamin A Level in the Blood in Young Rats Given Excess of Vitamin A, Compared with Normal Rats.

Conditions of experiment	Rat no.	Sex	Duration of experiment, days	Time, in hours, after last dose of vit. A until collection of sample	I.U. vit. A/g serum	Distinct symptoms of hypervitaminosis A
Basal diet (control)	2	♂	32	-	1.0	0
Basal diet + excess of vit. A. 10,000 I.U./day (96 I.U./g body weight)	1	♂	9	24	2.8	(0)
	2	♂	19	2 1/2	13.0	(0)
	3	♂	19	2	7.7	(0)
	4	♂	15	3	5.0	(0)
	5	♂	12	48	1.0	(0)
	6	♂	8	24	4.9	(0)
Basal diet + excess of vit. A, 50,000 I.U./day (576 I.U. vit. A/g body weight)	2	♂	32	335	0.25	+
	3	♂	20	24	4.6	+
	4	♂	20	24	3.2	+
Basal diet + excess of vit. A, 60,000 I.U. vit. A/day (730 I.U. vit. A/g body weight)	1	♂	9	0	3.8	+
	3	♂	13	0	1.8	+
	4	♂	14	3—4	13.5	+
	5	♂	12	24	13.4	+
	6	♂	8	0	19.0	+

Table 29.

Vitamin A Level in the Blood in Adult Rats Given Excess of Vitamin A.

Conditions of experiment	Rat no.	Sex	Duration of experiment, days	Time, in hours, after last dose of vit. A until collection of sample	I.U. vit. A/g serum	Distinct symptoms of hypervitaminosis A
Basal diet + excess of vit. A: 50,000—90,000 I.U./day, (260—480 I.U./g body weight)	1	♂	24	0	2.2	+
	2	♂	44	24	5.7	+
	3	♂	16	1/12	4.4	+
	4	♂	45	48	1.2	+
	5	♂	48	96	1.6	+
	6	♂	29	48	0.8	+
Basal diet + excess of vit. A: 50,000—90,000 I.U./day, (250—390 I.U./g body weight)	1	♂	46	2	17.0	+
	2	♂	46	3	15.6	+
	3	♂	46	4	9.4	+
	4	♂	13	24	4.2	+
	6	♂	46	1	9.4	+

Table 30.

Vitamin A Level in the Blood at Different Stages After the Last Dose of Vitamin A in Groups Given Various Doses of Vitamin A.

Time from last dose of vit. A until collection of sample, hours	I.U. vitamin A/g serum			
	10,000 I.U. vit. A/day	50,000 I.U. vit. A/day	60,000 I.U. vit. A/day	50,000—90,000 I.U. vit. A/day
0	-	-	19.0, 1.8, 3.8	2.2
1/12	-	-	-	4.4
1	-	-	-	9.4
2	7.7	-	-	17.0
2 1/2	13.0	-	-	-
3	-	-	-	15.6
3 1/2	5.0	-	14.0	-
4	-	-	-	9.4
24	4.9, 2.8	4.6, 3.2	13.4	5.7, 4.2
48	1.0	-	-	1.2, 0 8
96	-	-	-	1.6
335	-	0.25	-	-

A second group of six rats of the same weight as the previous group was given the same dose of vitamin A. In this case, one of the rats died at the end of 6 days, one was killed and examined at the end of 13 days, at which time the symptoms were pronounced, and the remaining four at the end of 46 days, at which time only one of these rats showed pronounced symptoms.

A third group of two rats of the same age and sex was used as a control, and was given the usual basal diet only. These rats remained free of any symptoms throughout the experiment. The results of the vitamin A determination in the blood are tabulated in table 29.

b) **The Vitamin A Content in the Internal Organs of Hypervitaminotic Rats.**

The results of vitamin A determinations of various organs in hypervitaminotic rats are compared with normal animals in tables 31 and 32. From these tables it is observed that the vitamin A content is largely increased in all internal organs examined in hypervitaminotic rats, such as the liver, kidney, adrenal, testis as well as the visceral fat. From table 31 it is evident that there is a considerable individual variation in the vitamin A content in the liver in rats within the same group.

In none of the cases was any vitamin A detected in the urine from hypervitaminotic rats, although the vitamin A content of the kidney was as high as 1,800 I. U. vit. A/g. No direct relation could be detected between the vitamin A content in the liver and the manifestation of clinical symptoms of hypervitaminosis A.

On the other hand it appears as if the most significant pathological changes are detected in the organs of hypervitaminotic animals where the storage of vitamin A is highest, such as in the liver and kidneys.

Table 31.

Vitamin A Contents of Livers in Hypervitaminotic Rats.

Conditions of experiment	Initial body weight, g	Duration of experiment, days	I. U. vit. A/g fat from liver	I. U. vit. A/g liver	Degree of clinical symptoms
Control.					
Basal diet	72	30	-	125	0
	68	33	10,000	406	0
	-	-	-	900	0
	Mean:		-	477	-
Basal diet + vit. A-free whale liver oil	76	30	-	800	0
Basal diet + excess of vit. A orally.					
25,000 I U. vit. A daily + vit. C	68	40	-	20,000	(+)
50.000 I.U. vit. A daily	87	84	55,000	3,474	+
50,000–90,000 I.U. vit. A daily	178	16	209,000	8,728	+ + +
	183	24	303,000	18,308	+ + +
	204	29	22,000	522	+ + (+)
	197	13	167,000	5,698	+ + +
	176	45	223,000	13,603	+ + +
	202	44	226,000	14,540	+ + +
	Mean:		172,100	10,610	-
Basal diet + single dose of vit. A.					
1.6 mill. I.U. vit. A	239	1	41,500	2,721	(+)
0.7 mill. I.U. vit. A	70	1	25,000	1,431	(+)
	Mean:		33,250	2,080	-
Basal diet + excess of vit. A given by subcutaneous injection.					
10,000 I.U. vit. A daily	100	33	62,000	5,320	+ +

0 = absence of clinical symptoms. (+) = very slight clinical symptoms. + = slight clinical symptoms. + + = moderate clinical symptoms. + + + = pronounced clinical symptoms.

Table 32.

Average Vitamin A Contents of Internal Organs in Adult Hypervitaminotic Rats.

Conditions of experiment	Daily dose of vitamin A, I.U.	Vitamin A content, I.U. vit.A/g			
		Adrenals	Kidneys	Testes	Visceral fat.
Basal diet (control)	20	33	1	1.5	2.5
Basal diet + excess of vitamin A	30,000	440	1800	120	-
	100,000	-	-	-	660

Summary of Results. It appears from these observations that there is no direct relation between the vitamin A level in the blood or the vitamin A content of the liver and the manifestation of clinical symptoms of hypervitaminosis A.

The vitamin A level in the blood may reach considerable values, without any symptoms being detected. Thus the vitamin A level was from 2.5—6.0 I. U./g, 24 hours after the last dose of vitamin A had been given both in rats given definite toxic doses (> 50,000 I. U./day), and in rats receiving doses of 10,000 I. U./day, which apart from a slightly reduced weight gain, produced practically no clinical symptoms.

A very rapid increase of the vitamin A level occurred up to 3 hours after the vitamin A dose had been given, followed by a rapid fall.

When 50,000 I. U., or more, of vitamin A was given daily, symptoms of acute intoxication usually occurred, in the form of drowsiness, inactivity, scruffiness etc., in which cases the vitamin A level of the blood was found to rise rapidly to about 14—17 I. U./g within 2—4 hours after the dose. When only 10,000 I. U. was given, the vitamin A level rose to 8—13 I. U., while the rat appeared unaffected as judged by clinical examination.

From these observations it may be concluded that the vitamin A concentration in the blood alone, or the vitamin A content of the liver, cannot be taken as an indicator of the condition of hypervitaminosis A in rats.

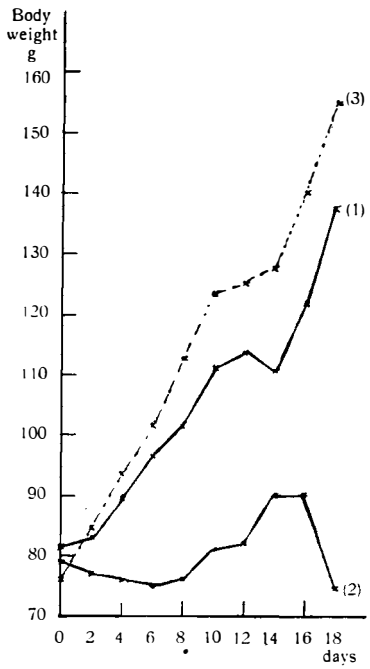
11. *Appetite, Food Consumption and Growth in Hypervitaminotic Rats.*

In previous experiments it was observed that the absence of the normal weight gain was one of the first and most constant indications of the effect of excess of vitamin A in rats.

In experiments it was possible to produce a distinct reduction in the weight increase without producing practically any of the other clinical symptoms which occurred when larger doses of vitamin A were given, and it was found that the greater the excess of vitamin A, the more marked was the reduction in the weight increase.

The weight curve was much more level in young growing rats when given excess of vitamin A, than in normal rats, — varying according to the magnitude of the overdosage with vitamin A. Adult rats lost weight as a result of massive doses of vitamin A. It was further shown that newborn rat babies from A-hypervitaminotic mothers, practically did not grow during the lactation period, when fed by the mother's milk, while normal weight gain occurred in the case when the milk was removed and replaced by the usual basal diet without any excess of vitamin A.

It might seem likely that the large quantities of whale liver oil given mixed in the diet, made the diet less palatable to the rats, resulting in a reduced food intake. The weight increase was affected, however,



Graph No. 9. Average weight graphs for rats given 10,000 I.U. vit. A daily (95 I.U. vit. A/g body weight) (1), and rats given 60,000 I.U. vit. A daily (730 I.U. vit. A/g body weight) (2), compared with normal rats (3).

whether the vitamin A oil itself was mixed in the diet, or whether it was given by pipet only, and no oil was mixed in the diet. Furthermore, the same effect was produced when highly purified sources of vitamin A were used, of which only four drops were given daily.

The question was then whether this absence of normal weight gain could be explained by a reduced food consumption, or whether other factors were at play.

If the reduced weight gain could be explained by the result of reduced food consumption, the next problem would be to decide whether the reduced food consumption could be attributed to the action of excess of vitamin A.

a) **Young Animals.**

In order to study the importance of the food consumption with regard to the growth of young hypervitaminotic rats, the food consumption was recorded in different groups of rats in connection with some of the previously described experiments.

In one experiment (Experiment 5, see page 33) a group consisting of two young rats with initial body weights of approximately 70—80 grams, were given whale liver oil, which originally had a potency of 200,000 I. U. per gram, but where the vitamin A had been destroyed by exposure to sunlight during a period of several weeks, at the end of which the antimony trichloride test, as well as spectrographical examination of the oil gave negative results. From this oil, 6 drops per rat were mixed daily with ordinary basal diet, and in addition to this

4 drops of the same oil were given to each rat daily by dropping pipet. In other words, 10 drops of this vitamin A-free whale liver oil were given to each rat daily, which would correspond to approximately 50,000 I. U. vitamin A before the vitamin A was destroyed. The control group consisting of two animals of the same weight, was given the ordinary adequate basal diet only.

In another experiment (Experiment 21, see pages 79 and 91) a group of 5 rats (initial weight 70—100 grams) was given the same whale liver oil containing all its vitamin A in the same quantities, and in the same manner as described for the first experiment, i. e. 6 drops per rat daily mixed in the diet, and 4 drops daily per rat by dropping pipet, — altogether 10 drops corresponding to 50,000 I. U. vit. A. In addition to this 1 mg aneurin per rat was given daily.

A second group consisting of 6 animals was given the same whale liver oil containing all its vitamin A in the following amounts: 6 drops per rat mixed in the diet, and 6 drops per rat by pipet daily, altogether corresponding to 60,000 I. U. per day per rat.

A third group was given two drops of whale liver oil per day per rat by pipet, and no whale liver oil was mixed in the diet. The daily dose of vitamin A for this group was thus 10,000 I. U. per rat.

A final group was used as a control, and was given the usual adequate basal diet only.

The food given to the rats was weighed every day at noon, and the remaining food was again weighed the following day at the same time, and the daily food consumption recorded. Simultaneously the milk consumption was recorded. Wasted food was carefully collected on a paper underneath the cage, weighed and deducted from the figures for the gross food consumption.

The food consumption was recorded during a period of 7 days after the mentioned doses had been given to the rats for about 10 days, and the results are given in table 33. The daily weight gain in these rats is recorded on page 34, graph no. 3, and in table 26 (page 80).

Table 33.

Food Consumption in Grams per Rat per Day During Seven Days in Young Rats.

Conditions of experiment	20/8—21/8	21/8—22/8	22/8—23/8	23/8—24/8	24/8—25/8	25/8—26/8	26/8—27/8	Mean
Control. Basal diet only	11.7	11.8	10.3	11.0	13.3	11.5	13.0	11.7
Basal diet + vit. A-free whale liver oil	11.7	12.8	11.0	13.5	12 8	15.3	9.3	12.2
Control. Basal diet only	12.5	14.8	12.8	16.0	14.5	13 4	18.0	14.5
Basal diet + whale liver oil, 60,000 I.U. vit. A/day	3.1	5.0	1.4	3.7	5.0	4.3	3.0	3.6
Basal diet + whale liver oil, 50,000 I.U. vit. A/day + 1 mg vit. B_1	6.1	6.7	4.8	5.4	6.0	5.9	7.0	5.9
Basal diet + whale liver oil, 10,000 I.U. vit. A/day	13.7	10.4	9.8	11.0	9.3	2.2	12.0	9.7

It will be seen from table 33 that the food consumption in the groups given the vitamin A-free whale liver oil was approximately the same, or slightly higher, than the control group (12.2 against 11.7) which shows that the whale liver oil itself, when mixed in the diet, does not cause a reduction of the appetite or the food intake when the vitamin A is destroyed.

In hypervitaminotic rats, on the other hand, there was a marked lack of appetite with a considerable reduction of the food intake, which was found to be in proportion to the given vitamin A doses, — that is the higher the dose of vitamin A, — the lower the food intake.

If the food intake was calculated in per cent of the normal consumption (the control group), the following relation between the vitamin A dose and the food consumption was found:

Table 34.

Relation Between Vitamin A Dose and Food Consumption.

Conditions of experiment	Vitamin A, I.U./day	Food consumption in 0/0 of the normal
Control, basal diet	20	100 0/0
Basal diet + 2 drops of whale liver oil	10,000	66.8 0/0
Basal diet + 10 drops of whale liver oil + 1 mg vit. B_1	50,000	40.6 0/0
Basal diet + 12 drops of whale liver oil	60,000	24.8 0/0

The milk consumption was approximately the same in the hypervitaminotic rats as in the normal rats.

At the end of the experiment, one of the rats in the group given 60,000 I. U. vitamin A daily, was taken out and kept separately in a cage and given ordinary basal diet only, without any excess of vitamin A, during the last twenty-four hours before it was killed. During those twenty-four hours it was found that the food consumption was only 2.5 grams, as against 3.6 grams during the experiment when excess of vitamin A was given mixed in the diet, which was still much less than the control group. This observation also supports the finding that the lack of appetite is not caused by the whale liver oil being mixed with the diet, but by the hypervitaminotic condition itself. Otherwise, if it is presumed that the adding of the oil to the diet would make the diet less palatable to the rats, one would have expected that the rat on basal diet would have consumed more than was the case, when the whale liver oil was mixed in the diet.

In order to study this further, two rats in the group which had previously received whale liver oil equivalent to 50,000 I. U. vitamin A per day (6 drops mixed in the food and 4 drops given by dropping pipet), and where a distinct lack of appetite, as well as absence of the normal weight gain was observed, — were removed, and given an identical amount of whale liver oil where the vitamin A had been destroyed by exposure to sunlight, i. e. 6 drops of this oil per rat daily mixed in the food, and 4 drops by pipet daily. The food consumption was recorded by daily weighing of the food as previously described. During the first 5 days of the experiment after the vitamin A had been removed from the diet, the

food consumption was 5.9 grams per day, during the second 5 day period it increased to 7.9 grams, and during the final 5 day period to 8.9 grams. It was observed that the daily food intake slowly increased from day to day, as the time went on, from 5 grams just after the vitamin A had been removed from the diet, to 10—12 grams at the end of 15 days, and at the same time an increase in the weight was observed.

b) **Adult Animals.**

In order to study the same conditions in adult rats, the food consumption was recorded in one group of 6 rats (see experiment 8, p. 38), which had an average weight at the beginning of the experiment of 193.8 g and which received 50,000 I. U. vitamin A daily (236 I. U./g body weight), i. e. 6 drops of whale liver oil per rat mixed in the basal diet, and 4 drops per rat given by a specially constructed catheter daily, during 12 days. At the end of this period the dose was increased to 90,000 I. U. daily.

For the purpose of comparison, the food consumption was recorded in a group of two rats with an average initial body weight of 201 g, which were given the usual basal diet only.

In this case the average food consumption in the two groups was recorded daily from the beginning of the experiment. The average daily food consumption in 5 day periods is recorded in table 35, and the weight graph is given on page 42.

In the first group receiving the vitamin A mixed in the adequate basal diet, the average daily food consumption was 8.6 grams during the first 5 days, as against 11.6 for the control group (74 % of the normal). The average daily food consumption remained fairly constant (70—74 % of the normal) during the first 20 days of the experiment, while towards the end of the experiment (25—35 days) it was only 55—56 % of the normal.

Simultaneously it was found that the milk consumption was higher in the control group compared with the hypervitaminotic rats.

Table 35.

Average Daily Food Consumption per Rat During 5-Day Periods in Adults Rats.

No. of days from beginning of the experiment	Basal diet (control)	Basal diet + whale liver oil, 50,000 I.U. vit. A/day. From the 12th day: 90.000 I.U. vit. A/day	
	Food consumption g/day	Food consumption g/day	Food consumption in % of the normal
0—5	11.6	8.6	74 %
5—10	13.8	9.8	71 %
10—15	13.7	10.1	74 %
15—20	15.2	10.9	72 %
25—30	14.2	7.8	55 %
30—35	19.1	10.8	56 %
Mean	14.6	9.7	67 %

In a final group of 6 adult rats (see experiment 8, p. 38) which were given a similar dose of vitamin A by a specially constructed catheter, the average daily food consumption was approximately 90 % of the normal during the first 20 days. During the last 5 days period of the experiment, however, from the 30th—35th day, when the dose had been increased to 90,000 I. U. vit. A daily, the food consumption was only 42 % of the normal.

In this experiment the food consumption was higher in adult rats receiving the vitamin A oil by catheter as compared with the group given the oil mixed in the diet. In this connection it may be noted that the symptoms of hypervitaminosis A were generally found to be more pronounced and occurred earlier in the cases where the oil was partly given mixed in the diet, than when given by catheter or dropping pipet only. Furthermore, it was found that the loss of vitamin A through feces was approximately 20 % higher in rats given the vitamin A oil by catheter, as compared with rats receiving the oil mixed in the diet.

From these experiments it appears that the lack of appetite and the reduced food consumption is less in adult rats as compared with the young rats, when given the same daily gross dose of vitamin A. When the same dose of vitamin A per gram body weight is given, however, to young and adult rats, there is a more reasonable agreement between the doses of vitamin A per gram body weight and the effect on the appetite and food consumption in both cases.

Summary of Results. From these experiments it may be concluded that the reduced weight increase may well be explained as the result of reduced food intake in the hypervitaminotic rat, and that the lack of appetite observed in hypervitaminotic rats is caused by the action of vitamin A. It was found that whale liver oil when given to rats had no adverse effect on the appetite or the weight increase when the vitamin A was destroyed.

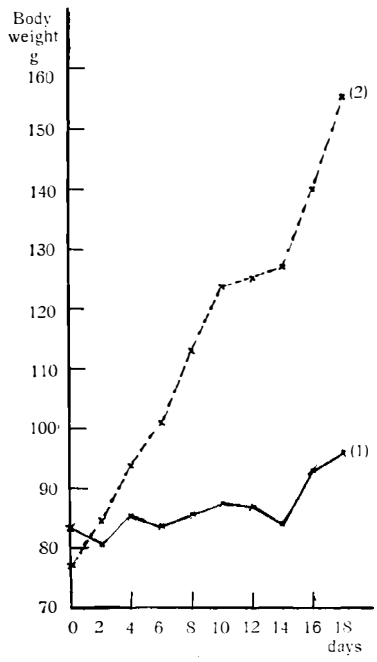
12. *Hypervitaminosis A and Vitamin B_1.*

It has been reported by previous workers (Harris & Moore, 1928; and Bell, Gregory & Drummond, 1933) that additional allowance of the vitamin B complex has a beneficial effect on animals given excess of marine oil, while other workers (Moore & Wang, 1945) failed to find any obvious effect on the development of hypervitaminosis A by varying the doses given of the vitamin B complex.

The purpose with the following experiment was to investigate whether additional supply of large doses of vitamin B_1 had any effect on the observed absence of normal weight gain or any of the other symptoms in rats fed on massive doses of vitamin A.

Experiment 21.

For this purpose a group of 5 rats with an average initial body weight of 83.7 grams (68.0—99.5), were given 50,000 I. U. vitamin A daily in the form of 10 drops of whale liver oil concentrate (6 drops of the oil per rat mixed in the basal diet, and 4 drops of the same oil given daily by dropping pipet). This dose corresponded to an average of 570 I. U. vit. A/g body weight daily. In addition to this 1 mg of aneurin (vit. B_1) was given daily, mixed in the basal diet. The food mixture was prepared fresh daily.



Graph No. 10. Average weight graphs for rats given 570 I. U. vit. A/g body weight + 1 mg vitamin B_1 daily (1), compared with normal rats (2).

For control purposes a group of two normal rats with an average initial body weight of 76.7 grams (67.5—86.0), were given the usual basal diet only.

Some of the details of this experiment have already been described in chapters 10 and 11 (see pages 79 and 86).

The mentioned dose of vitamin A produced a considerable reduction of the weight gain, and distinct symptoms of hypervitaminosis A in the group receiving additional supply of vitamin B_1. The average daily weight gain during the first 10 days of the experiment was 0.4 g and during the first 18 days it was 0.7 g, — as against 4.7 g daily during the first 10 days, and 4.4 g daily during the first 18 days in the control group (see graph no. 10). The food consumption was only approximately 40 % of the normal (see tables 33 and 34).

In all cases the rats appeared scruffy; there was weakness and muscular atrophy. Soreness around the eyes and nose was observed in all cases. Alopecia was observed on the head and around the anus in one of the rats, and diarrhea in three. Swelling of the palpebrae was observed in one case. Fractures were clinically diagnosed in all rats, and in four of these there were fractures of all four extremities. In one case the dose proved lethal at the end of 15 days.

The control rats remained free of any symptoms throughout the experiment, except for soreness around the eye in one of the rats, which lasted a few days.

X-ray examination of the long bones in the control group revealed normal conditions, while in the group given excess of vitamin A and vitamin B_1 the same findings were revealed as previously described for the hypervitaminotic rats. Around the fractures there was marked callus formation in spite of the fact that the vitamin A had been given continuously in unchanged doses (ill. 18).

The blood coagulated within 5 minutes, and there was no deminite anemia (hemoglobin: 82, 90, 93 and 102 %, as against 104 % in the control group).

The ascorbic acid content of the liver and adrenals was determined in one rat, the figures being 8 and 72 mg/100 g respectively.

Postmortem examination revealed the following findings: In one case there were hemorrhages around the elbow, but otherwise there were no subcutaneous or visceral hemorrhages except for the hemorrhages around the fractures. The livers appeared fatty on the cut surface and the adrenals appeared enlarged, which was verified by careful weighing (0.021—0.043 % of the body weight, as against 0.013 % in the control). In one case the spleen appeared considerably enlarged. Histologically, similar findings were made as previously described for the hypervitaminotic rats (see page 82).

By postmortem examination of the control group, normal organs were found, except for an enlarged spleen and several abscesses in one of the kidneys in one case.

Summary of Results. It may be concluded from this experiment that additional supply of vitamin B_1 had no effect on the appetite and weight gain in hypervitaminotic rats, nor did it have any influence on the symptoms of hypervitaminosis A.

13. *Hypervitaminosis A and Vitamin D.*

Bomskov and Seeman (1933) state that the condition of hypervitaminosis A counteracted the ricket-curing capacity of vitamin D.

In connection with the present investigations it was examined whether lack of vitamin D had any effect on the clinical picture of hypervitaminosis A, — and whether excess of vitamin A interfered with the development of rickets in rats kept on a rachitic diet, and finally whether excess of vitamin A hindered the ricket-curing capacity of vitamin D.

In the following investigations the X-ray method for diagnosis of rickets was applied. The ash and mineral content of the bones was determined in a number of cases at various stages of the experiment. As a rachitic diet the Shermann-Pappenheimer's diet was used with the following composition:

egg albumin	(5 %)	250 g
$CaCO_3$	(2 »)	100 »
NaCl	(2 »)	100 »
ferrous chloride	(0.2 »)	10 »
wheat flour	(90.8 »)	4540 »

In additions to this 3 g spinach was given to each rat per week.

a) **Preliminary Investigations.**

Experiment 22.

In a preliminary experiment excess of vitamin A in the form of whale liver oil concentrate was given to a group of rachitic rats, which was continuously kept on the rachitic diet without vitamin D. Doses of 2, 4, 8 and 10 drops of the whale liver oil were given daily during a period of 10 days, corresponding to 10,000; 20,000, 40,000, and 50,000 I. U. vit. A daily, or 110, 285, 500, and 550 I. U./g body weight. In all cases except the first, pronounced symptoms of hypervitaminosis A developed during this period. As judged by the clinical symptoms it appeared as if the symptoms of hypervitaminosis A occurred earlier and were more pronounced in rachitic rats than in rats given the usual adequate basal diet. In agreement with this, doses of vitamin A which normally did not cause any clinical symptoms of

hypervitaminosis A caused distinct hypervitaminotic symptoms in rachitic rats. Thus in a further group of 4 rachitic rats, approximately 2,500 I. U. vit. A daily, corresponding to 40 I. U./g body weight, caused symptoms of hypervitaminosis A with weakness, limping, alopecia, soreness of the skin as well as swelling of the palpebrae. The results of blood examinations in this group are given in table 36. From this table it is seen that there was distinct anemia in one case, and the ascorbic acid content of the serum was abnormally low in two cases.

Table 36.

Results of Blood Examinations in Rachitic Rats Given 2,500 I. U. Vit. A Daily (40 I. U. Vit. A/g Body Weight).

Rat no.	Sex	Hb %	mg ascorbic acid/100 ml serum
1	♀	92	0.62
2	♂	101	0.35
3	♀	98	0.25
4	♀	65	0.50
Mean		89	0.43

b) **The Effect of Excess of Vitamin A in Rats Given a Rachitic Diet.**

Experiment 23.

A group of 4 rats was given 50,000 I. U. vit. A daily (740 I. U. vit. A/g body weight) in the form of whale liver oil concentrate in addition to the rachitic diet during a period of 18 days.

Already from the 4th day distinct symptoms of hypervitaminosis A were detected, — such as scruffiness, weakness, decreased activity, limping, as well as eye symptoms and alopecia.

All rats developed rickets similar to rats given rachitic diet only. On the other hand the combination of excess of vitamin A and a rachitic diet was more injurious to the rats than when excess of vitamin A was given in addition to the usual adequate basal diet, — the symptoms of hypervitaminosis A occurring more quickly and being more pronounced in the first case.

Additional supply of vitamin D in addition to the rachitic diet produced healing of the rickets as judged by the roentgenograms at the end of 10 days, in spite of the excess of vitamin A being given continuously in unchanged doses, while the symptoms of hypervitaminosis A progressed with increased weakness, fractures, and macroscopical hematuria as well as intestinal bleeding. In the urine the Heller's test was positive, and by microscopical examination of the centrifuged urine a large number of red cells were seen per field of vision.

Although X-ray examination revealed healing of the rickets, the ash content of the bones was less than in normal rats, but similar to other rachitic rats given vitamin D during a similar period without excess of vitamin A. The calcium and phosphorus contents of the ash, however,, were not reduced (see table 38).

Two of the rats were continously given excess of vitamin A in addition to the rachitic diet and additional supply of vitamin D, — one during a period of 20 days and one 68 days. During these periods the weights remained practically unchanged, and the symptoms of hypervitaminosis A gradually progressed, as well as multiple fractures and marked bone deformities.

At autopsy emaciation and fatty appearance of the cut surface of the liver was observed in all cases, swelling of the costochondral junction and deformities of the thorax in two cases, large deposits of pigment around the kidneys and thymus and along the aorta and ribs, as well as free blood in the knee joints in one case. In two cases the pancreas appeared enlarged.

Histologically, hyperemia was revealed in the internal organs in the two rats examined. A few red blood cells were seen in the space of Bowman's capsule in the kidney. In the liver stained by sudan III, considerable deposits of sudanophil droplets were seen throughout the organ, and in the adrenal, slight sudanophil deposits throughout the cortex. No fatty degeneration was detected in the kidney.

The results of blood examinations are tabulated in table 37.

Table 37.

Results of Blood Examination in Rats Given Excess of Vitamin A + Vitamin D in Addition to Rachitic Diet.

Rat no.	Sex	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	mg ascorbic acid /100 ml serum
2	♂	79	4.4	0 9	-
3	♂	88	5.5	0.8	0.73
4	♂	68	4.4	0.8	-

Table 38.

Ash and Mineral Contents of the Femurs in Rats Given Excess of Vitamin A + Vitamin D in Addition to Rachitic Diet.

Conditions of experiment	Rat no.	Sex	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Rachitic rats given vit. D 12 days, without excess of vit. A	2	♀	28.3	24.6	22.1	22	2	0
Rachitic diet + vit. D + excess of vit. A (50,000 I.U. daily)	2	♂	33.6	31.4	23.1	23	2	+
	3	♂	28.2	32.9	18.0	23	2	+
	Mean:		30.9	32.2	20.5	23	2	-

Experiment 24.

A group of 5 rats was given 50,000 I. U. vit. A daily in the form of whale liver oil in addition to the rachitic diet. This dose proved lethal in one of the rats at the end of 7 days, in three at the end of 11 days, and one at the end of 13 days. In all cases the symptoms of hypervitaminosis A were very pronounced, with scruffiness, weakness, soreness in the skin, fractures, alopecia, eye symptom and massive hematuria. In all cases the rats lost weight.

X-ray examination revealed only slight rickets, while there were multiple fractures and bone changes typical of hypervitaminosis A. The ash contents of

the femurs were less than in normal rats, while the calcium and phosphorus contents of the ash were not reduced (see table 39). The hemoglobin was 88 and 85 % in the two cases examined.

Postmortem examination revealed: hyperemia, scattered small subcutaneous hemorrhages, enlarged adrenals, fiery red lungs without any signs of pneumonia, and brittle bones. In one case the pancreas appeared enlarged and fiery red, and in one case there was free blood in both knee joints. In one case the left adrenal weighed 0.012 g, i. e. 0.032 % of the body weight.

Histological examination of the liver, kidneys, adrenals, pancreas, lungs, bones and teeth in three of the rats revealed hyperemia in the internal organs, scattered red blood cells in some of the spaces of Bowman's capsules in the kidney in two of the rats, and slight degeneration of the renal tubules in one. In the liver stained by sudan III, dense deposits of sudanophil droplets were seen throughout the organs, particularly in the Kupffer cells, which appeared swollen. In the adrenal sudanophil deposits of moderate density were seen throughout the cortex. In the teeth, there was marked hyperemia in the pulp, which in one case contained vacuoles and deposits of a calcium-like substance. The odontoblasts were irregularly arranged (see ill. 51). In the tibia there was great irregularity of bone structure, the compact bone being very irregularly developed, and very thin in some places, and showed fractures. There were signs of increased osteoclastic activity, and scattered red blood cells were seen outside the capillaries in the connective tissue.

Table 39.

Ash and Mineral Contents of the Femurs in Rats Given 50,000 I. U. Vitamin A Daily in Addition to Rachitic Diet.

Rat no.	Sex	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
1	♂	11	45.3	34.3	17 6	19	1.5	+
3	♀	7	40.1	35.8	21.1	-	-	+
5	♀	11	44.5	35.7	19.1	18	1.5	+
Mean:			43.3	35.3	19.3	19	1.5	-

Experiment 25.

In a further experiment two rats were given excess of vitamin A (50,000 I. U. daily) and vitamin D in prophylactic doses, in addition to the rachitic diet from the beginning of the experiment.

At the end of 16 days no rickets were detected, while the rats suffered from pronounced symptoms of hypervitaminosis A, with marked eye symptoms, weakness, alopecia, soreness, fractures, large subcutaneous hemorrhages, and roentgenological bone changes, typical for hypervitaminosis A. In one case the dose of vitamin A proved lethal at the end of 28 days. In this case postmortem examination revealed also hemorrhages in the skin over the abdomen, hyperemia, swollen visceral lymph glands, and diffused intestinal hemorrhage.

In a second rat the hemoglobin was 85 %, the serum iron: 111 γ %, and the fasting blood sugar: 156 mg/100 ml.

c) The Effect of Excess of Vitamin A in Rachitic Rats.

Experiment 26.

A group of 4 rachitic rats was given 50,000 I. U. vit. A daily in the form of whale liver oil concentrate, as well as vitamin D in curative doses, in addition to the rachitic diet during periods varying from 22 to 72 days.

Distinct symptoms of hypervitaminosis A were detected on the 4th day in all cases, with exophtalmus and limping (ill. 4). Curing of the rickets occurred, however, in all the rats at the end of 10 days. Increasing weakness occurred in all cases from the 16th day, and fractures on the 20th day, at which time the general condition was very poor, with soreness and marked bone deformities, and in two cases the rats had lost the use of their legs. In one case there was bleeding in the skin around the knees, and priapismus which lasted from the 48th day of the experiment, until the rat was killed on the 72nd day (see ill. 6). Marked alopecia was observed in two cases. There was practically no weight increase throughout the experiment.

The blood was examined in one case on the 40th day of the experiment, and again at the conclusion of the experiment on the 72nd day with results as shown in table 40.

Table 40.

Results of Blood Examinations in a Rachitic Rat Given Excess of Vit. A, at the End of 40 and 72 Days.

No. of days from the beginning of the experiment, until collection of sample	Hb %	Red blood cells, millions per 1/1000 ml	Colour index
40	90	5.6	0.9
72	65	4.6	0.7

It must be noted that after the first blood sample was examined, bleeding in the skin around the knees and multiple fractures with muscular hemorrhages were detected in this rat. At the conclusion of the experiment, the serum iron in this rat was 35 γ % determined by Heilmeyer and Plötner's method.

The results of blood examinations of all rats in this group are given in table 41. From this table it is observed that the ascorbic acid content of the serum was abnormally low in the two cases examined.

Postmortem examination revealed general hyperemia, swollen visceral lymph glands, and swelling of the costochondral junction. The liver was fatty on the cut surface, and the bones were brittle. In one case the pancreas appeared enlarged, and in two cases the spleen appeared enlarged. Large deposits of pigment were observed along the aorta and around the thymus in one case.

Microscopical examination of the liver, kidneys, adrenals and pancreas in two of the rats revealed hyperemia, slight degeneration of the renal tubules, and a few red blood cells in the space of Bowman's capsule in one case. In the liver stained by sudan III, massive deposits of sudanophil droplets were seen througout the organ, both in and between the liver cells. In the adrenal abnormally dense sudanophil deposits were seen in the cortex, particularly in the zona glomerulosa.

Table 41.

Results of Blood Examinations in Rachitic Rats Given Vitamin D + Excess of Vitamin A (50,000 I. U. Daily).

Rat no.	Sex	Hb %	Red blood cells, millions per 1/1000 ml	Colour index	Serum iror, γ %	mg ascorbic acid /100 ml serum	Fasting blood sugar
1	♂	100	-	-	-	-	109
2	♀	70	4.6	0.8	-	0.13	-
3	♂	65	4.6	0.7	35	-	-
4	♀	79	-	-	-	0.18	-

Although healing of the rickets had occurred as judged by X-ray examination, the ash contents of the bones in these rachitic rats which had received excess of vitamin A in addition to vitamin D and the rachitic diet during 22—72 days, were less than in normal rats, as is evident from table 42. The calcium and phosphorus contents of the ash were above normal, however.

Table 42.

Ash and Mineral Contents of Femurs in Rachitic Rats Given Excess of Vitamin A in Addition to Vitamin D.

Conditions of experiment	Rat no.	Sex	No. of days on vit. D + excess of vit. A	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Rachitic rats given vit. D + excess of vit. A (50,000 I.U. daily)	1	♂	22	23.5	45.2	27.2	-	-	+
	2	♀	70	30.0	42.6	22.8	22	1.9	+
	3	♂	72	43.4	30.3	19.1	27	2.0	+
	4	♀	72	44.7	33.6	19.3	25	1.5	+
	Mean:			35.4	37.9	22.1	25	1.8	-

Experiment 27.

A group of four rachitic rats were given 50,000 I. U. vitamin A daily during a period of 12 days, in the form of whale liver oil, in addition to the rachitic diet + 1 I. U. vit. D daily, after distinct rickets had been diagnosed. At the end of this period they were killed and examined, at which time symptoms of hypervitaminosis A were pronounced, but complete healing of the rickets as judged by roentgenograms had taken place. The ash and mineral contents of the femurs are given in table 43.

Table 43.

Ash and Mineral Contents of the Femurs in Rachitic Rats Given Excess of Vitamin A (50,000 I. U. Daily) + 1 I. U. Vit. D Daily During 12 Days.

Rat no.	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
1	37.0	27.6	24.4	20	2.0	0
2	42.2	33.6	19.2	24	2.0	0
3	33.7	33.0	25.0	22	2.2	0
4	30.7	39.2	22.5	22	2.0	0
Mean:	35.9	33.4	22.8	22	2.0	-

Microscopical examination of the liver, kidney, adrenal and pancreas in one of these rats revealed similar findings as previously described for the hypervitaminotic rats.

Experiment 28.

A group of five rachitic rats received 80,000 I. U. vitamin A daily in the form of whale liver oil in addition to vitamin D and rachitic diet. Although they all suffered from marked symptoms of hypervitaminosis A, — which in two cases were fatal, complete healing took place of the rickets in all cases as judged by roentgenograms. The ash content of the femurs was less than normal, however, but similar to that of rachitic rats which had received vitamin D during a period of 12 days without excess of vitamn A (see table 44).

Marked and increasing weakness, scruffiness, limping, diarrhea and alopecia occurred in all cases after three days. In all cases fractures occurred and the rats appeared to have lost the use of their hind legs. In one of the rats which died, dyspnoea was observed.

Table 44.

Ash and Mineral Contents of the Femurs in Rachitic Rats Given Excess of Vitamin A, and Vitamin D.

Conditions of experiment	Rat no.	Sex	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Rachitic rat given vit. D 12 days	2	♀	28.3	24.6	22.1	22	2.0	0
Rachitic rats given vit. D + excess of vit. A (80,000 I. U. vit. A daily) 8—10 days	1	♀	27.3	38.1	23.3	21	1.9	+
	2	♀	28.7	44.3	25.3	20	2.1	+
	3	♂+♀	29.7	27.6	-	-	-	+
	4	♂	31.3	40.0	27.2	-	-	+
	Mean:		29.3	37.5	25.3	21	2.0	-

Table 45.

Manifestation of Clinical Symptoms in Rats Given Excess of Vitamin A in Addition to Rachitic Diet.

Conditions of experiment	Rat no.	Sex	Lethal result	Reduced weight gain	Weakness	Lack of activity	Scruffiness	Alopecia	Soreness of skin	Swelling of palpebrae	Exophtalmus	Diarrhea	Limping	Fractures	Pathological X-ray findings	Hemorrhages	Priapismus
Rachitic diet + vit. D																	
50,000 I.U. vit. A	1	♂		+	+	+	+		+		+		+	+	+		
daily (550—600	2	♀♂	+	+	+	+	+		+		+		+	+	+		
I.U./g body weight)	3	♂	+	+	+	+	+	+	+	+	+		+	+	+	+	+
	4	♀		+	+	+	+	+	+		+		+	+	+		
50,000 I.U. vit. A	1	♂	+	+	+	+	+	+	+	+			+	+	+		
daily (740 I.U./g	2	♂	+	+	+	+	+	+	+	+			+	+	+		
body weight)	3	♂	+	+	+	+	+			+			+	+	+		
	4	♂	+	+									+		+		
50,000 I.U. vit. A	1	♀		+	+			+	+	+	+		+	+	+	+	
daily (500—1000	2	♀	+	+	+			+		+	+		+	+	+	+	
I.U./g body weight)																	
80,000 I.U. vit. A	1	♀	+	+	+	+	+	+	+			+	+	+	+	+	
daily (1000 I.U./g	2	♀♂	+	+	+	+	+	+	+			+	+	+	+		
body weight)	3	♂	+	+	+	+	+	+	+			+	+	+	+	+	
	4	♂	+	+	+	+	+	+	+			+	+	+	+	+	
	5	♂	+	+	+	+	+	+	+	+		+	+	+	+	+	
Rachitic diet without vit. D																	
50,000 I.U. vit. A	1	♂	+	+	+	+	+	+	+	+	+		+	+	+	+	
daily (1000 I.U./g	2	-	+	+	+	+	+	+	+	+	+		+	+	+	+	
body weight)	3	♀	+	+	+	+	+	+	+	+	+		+	+	+	+	
	4	-	+	+	+	+	+	+	+	+	+		+	+	+	+	
	5	♀	+	+	+	+	+	+	+	+	+		+	+	+	+	

By postmortem examination, the following pathological findings were made: emaciation, hyperemia, swelling of the costochondral junction, fatty cut surface of the liver in all cases, — free blood in the knee-joints in 4 cases, swelling of the visceral lymph glands and large deposits of pigment along the aorta in three, scattered visceral and subcutaneous hemorrhages in two, and hemorrhages on the surface of the brain in one case. In one rat the pancreas and lungs were fiery red.

Blood examination revealed slightly reduced hemoglobin in the rachitic rats given vitamin D and excess of vitamin A, the values being 79—88 %, as against 99 % in rachitic rats given vitamin D without excess of vitamin A. The ascorbic acid content of the serum was abnormally low in the rachitic rats given excess of vitamin A in addition to vitamin D (0.00—0.26 mg/100 ml serum).

Summary of Results from Experiments 22—28. From these experiments it is evident that the condition of hypervitaminosis A did not effect the development of rickets in rats kept on a rachitic diet, nor did it hinder the protective effect of vitamin D given prophylactic to rats kept on a rachitic diet. Finally excess of vitamin A did not prevent the ricket-curing effect of vitamin D in rachitic animals, as judged by the roentgenograms, although the ash contents of the bones in these cases did not rise to normal values.

On the other hand, excess of vitamin A was more injurious to rats given a rachitic diet, than to rats given the usual adequate basal diet.

14. *Hypervitaminosis A and Vitamin K.*

Light, Alscher and Frey (1944) found that hypervitaminosis A in rats was associated with a pronounced hypoprothrombinemia, which could be corrected by giving vitamin K. This observation has been confirmed by Walker, Eylenburg and Moore (1947), who concluded that a secondary deficiency of vitamin K may be induced by toxic excess of vitamin A, although any satisfactory explanation of the action of vitamin K in this case has not been given.

In a previous investigation (Rodahl, 1949, 2) additional supply of vitamin K was found to have no influence on the incidence of hemorrhages or any of the other symptoms in hypervitaminotic rats.

Experiment 29.

In a further experiment a group of 6 adult rats were given 0.5 g daily of the liver from a hypervitaminotic dog, containing 90,000 I. U. vitamin A per gram (45,000 I. U. vit. A daily), mixed in the usual basal diet during a period of 14 days, at which time they all showed distinct symptoms of hypervitaminosis A. They were then fasted 24 hours, receiving only water, — at the end of which period they were all killed in

Table 46.

Prothrombin Time in Adult Rats Given 45,000 I. U. Vit. A Daily During 14 Days.

	Prothrombin time, seconds.
Hypervitaminotic rats no. 1	14.7
„ 2	14.0
„ 3	18.6
„ 4	16.9
„ 5	18.6
„ 6	15.0
Normal control	14.0—16.0

the usual manner after blood samples had been collected for the determination of the prothrombin time by the method described on page 21. The results are given in table 46, from which it is observed that in only two out of 6 rats the prothrombin time was prolonged.

Determination of the prothrombin time was also carried out in a number of animals in the present investigation by the same method, the results of which are summarised in table 47. From this table it is evident that it in a number of cases the prothrombin time is increased in the hypervitaminotic rats. There appears to be no direct relationship between the magnitude of the overdosage with vitamin A and the prothrombin time, however.

In some cases increased prothrombin time was associated with anemia, but in no case with hemorrhages. On the other hand hemorrhages were detected in two cases when the prothrombin time was normal. It is thus evident that the hemorrhages in these cases cannot be explained by hypoprothrombinemia.

Table 47.

Prothrombin Time in Hypervitaminotic Rats in Relation to Serum Colour, Hemoglobin, and Degree of Clinical Symptoms.

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Hemorrhages	Degree of symptoms 0 = none + = slight + + = moderate + + + = marked	Hb %	Serum colour (Meulengracht's)	Prothrombin time, seconds
Basal diet (control)	♀	87	28	0	0	105	3	19.0
	♂	45	23	0	0	100	3	15.5
	♀	Adult	-	0	0	-	-	16.0
	♂	Adult	-	0	0	93	-	17.0
	Mean:		-	-	-	99	3	16.9
Basal diet + excess of vit. A								
25,000 I. U. vit. A daily	♀	178	30	0	+ + +	67	3	14.1
37,500 I. U. vit. A daily	♀♂	78	58	+	+ +	78	3	15.0
50,000 I. U. vit. A daily	♂	87	84	0	+	76	10	25.0
— — —	♀	102	119	0	+ +	86	10	22.5
— — —	♀	83	119	0	+ +	74	12	26.5
— — —	♀	238	55	0	+	-	-	22.0
— — —	♂	210	55	0	+ +	-	-	16.0
— — —	♀	47	75	0	+	74	12	26.5
60,000 I. U. vit. A daily	♀	91	28	+	+ +	93	3	16.5
— — —	♀	84	42	0	+ + +	58	-	24.0
	Mean:		-	-	-	76	8	20.8
Basal diet + excess of vit. A + vit. C								
50,000 I. U. vit. A daily	♀	50	20	0	+	90	3	15.0
60,000 I. U. vit. A daily	♀	89	42	0	+	74	3	17.0
	Mean:		-	-	-	82	3	16.0

In the majority of cases increased prothrombin time was associated with increased serum colour as determined by Meulengracht's test.

In all cases when large doses of vitamin K were given 24 hours before the blood sample was collected, the prothrombin time was normal (see table 20). It must be noted, however, that in one of these cases, it was also normal before vitamin K was given.

From these observations it is evident that it is necessary to look for other factors than vitamin K deficiency in order to explain the tendency to bleeding in hypervitaminotic rats.

15. *Hypervitaminosis A and Vitamin C.*

The similarity between the clinical picture of hypervitaminosis A in rats, and that of scurvy has been pointed out by Vedder and Rosenberg (1938) who found that ascorbic acid offered protection against excess of vitamin A, — and by Moore and Wang (1945) who failed, however, to detect any abnormality in the ascorbic acid metabolism in their hypervitaminotic rats, or to produce any beneficial effect of 50 mg ascorbic acid daily, when mixed in the basal diet, in rats receiving excess of vitamin A.

As is evident from the present investigation, the clinical and post-mortem findings in our hypervitaminotic animals have produced further evidence of the similarity between hypervitaminosis A and scurvy.

A detailed study of the condition of hypervitaminosis A with this in view, as well as an examination of the vitamin C contents and the effect of additional supply of vitamin C in hypervitaminotic rats, was therefore considered desirable.

a) **The Vitamin C Contents in Hypervitaminotic Rats.**

1. *Liver and Adrenals.*

The results of vitamin C determinations in the liver and adrenals in hypervitaminotic rats in the previously described experiments are summarized in table 48. From this table it appears evident that the average vitamin C contents of the liver and of the adrenals are lower in rats given massive doses of vitamin A than in normal control animals. On the other hand the vitamin C contents of the adrenals were higher than with normal control animals when only 10,000 I. U. vitamin A was given daily.

2. *Blood and Urine.*

The results of vitamin C determinations in the serum in hypervitaminotic as well as normal control rats in the various experiments described in this paper, are summarized in table 49.

From this table it appears evident that the average vitamin C content of the serum is lower in hypervitaminotic rats than in normal rats, or in rats given the liver oil freed of its vitamin A content. Moderate excess

Table 48.

Ascorbic Acid Contents of the Liver, and Adrenals in Hypervitaminotic Rats.

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Ascorbic acid content, mg/100 g	
				Liver	Adrenal
Basal diet (control)	♂	233	50	25	125
	♂	169	50	22	103
	-	-	-	21	350
	Mean:	-	-	23	193
Basal diet + excess of vit. A					
10,000 I.U. vit. A daily	♀	67	58	18	-
	♂	83	58	18	-
	♀	77	58	12	-
	♂	68	9	27	320
	♂	87	19	20	400
	♂	96	19	28	400
	♂	78	15	22	240
	♂	85	12	25	480
	♂	85	12	17	373
	Mean:	-	-	21	358
25,000 I.U. vit. A daily	♀	160	19	-	60
50,000 I.U. vit. A daily	♂	68	15	8	72
60,000 I.U. vit. A daily	♂	69	9	8	213
	♂	95	18	2	100
	♂	99	13	6	85
	♂	80	14	17	200
	♂	68	12	10	140
	♂	67	8	8	200
	Mean:	-	-	9	156
50,000—90,000 vit. A daily	♂	202	44	8	69
	♂	176	45	11	90
	♂	220	48	21	20
	♂	213	46	20	49
	♂	223	46	17	40
	♂	150	46	19	72
	♂	216	46	18	59
	Mean:	-	-	16	57
100,000 I.U. vit. A daily	♂	163	46	12	-
	♂	192	46	16	-
	♀	147	28	19	19
	♀	170	46	8	-
	♀	161	46	6	-
	♀	143	46	18	-
	Mean:	-	-	13	19

Table 49.

Results of Vitamin C Determinations in the Serum of Normal, and Hypervitaminotic Rats.

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	mg ascorbic acid per 100 ml serum
Basal diet (control)	♂	49	95	0.82
	♀	50	26	0.86
	♀	87	28	0.70
	♀	93	33	0.50
	♂	64	24	0.54
	♂	233	50	0.70
	♂	169	50	0.56
	♂	216	11	0.56
	♀	21	73	0.93
	♀	26	73	0.42
	♂	38	73	1.15
	-	-	-	0.65
	Mean:	-	-	0.67
Basal diet + vit. A-free liver oils	♂	59	29	0.94
	♂	59	29	0.79
	♂	104	11	0.60
	♂	96	11	1.05
	Mean:	-	-	0.80
Basal diet + moderate excess of vit. A				
10,000 I. U. vit. A daily	♀	67	58	0.56
	♂	83	58	0.78
	♀	77	58	0.43
	♀	69	53	0.83
	Mean:	-	-	0.65
13,000—15,000 I. U. vit. A daily	♂	45	100	0.80
	♀	46	97	0.42
	Mean:	-	-	0.61
Basal diet + excess of vit. A in toxic doses				
15,000 I. U. vit. A daily	♀	44	26	0.26
	♀	49	26	0.18
	♀	53	26	0.32
	Mean:	-	-	0.25
25,000 I. U. vit. A daily	♀	174	34	0.29
	♂	170	34	0.46
	♂	176	34	0.71
	♀	166	34	0 29
	Mean:	-	-	0.44
30,000 I. U. vit. A daily	♀	210	40	0.43
	♀	152	40	0.45
	Mean:	-	-	0.44
37,000 I. U. vit. A daily	♂	86	45	0.36
	♂	85	45	0.33
	Mean:	-	-	0.34

Table 49 (cont.)

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	mg ascorbic acid per 100 ml serum
Basal diet + excess of vit. A in toxic doses (continued)				
50,000 I. U. vit. A daily	♂	90	65	0.74
	♂	81	65	0.85
	♂	139	69	0 59
	♀	102	119	0.08
	♀	83	119	0.13
	♂	87	84	0.13
	♂	61	27	0.00
	Mean:	-	-	0.36
52,500 I. U. vit. A daily	♀	60	21	0.39
60,000 I U. vit. A daily	♀	80	38	0.18
	♀	91	28	0.05
	Mean:	-	-	0.11
50,000—90,000 I. U. vit. A	♂	202	44	0.37
	♂	176	45	0.49
	♂	220	48	0.32
	♂	203	46	0.45
	♂	223	46	0.46
	♂	150	46	0.41
	♂	216	46	0.20
	Mean:	-	-	0.39
100,000 I. U. vit. A daily	♀	170	46	0.13
	♀	143	46	0.45
	Mean:	-	-	0.29
Excess of vit. A given by subcutaneous injection				
10,000 I. U. daily	♀	100	33	0.28
1,4 mill. I. U. in single injection	♂	186	11	0.49
Rachitic diet + excess of vit. A				
2,500 I. U. vit. A daily	♀	57	13	0.62
	♀	70	13	0.25
	♀	51	13	0.50
	Mean:	-	-	0.46
50,000 I. U. vit. A daily + vit. D	♀	57	72	0.13
	♀	74	72	0.18
	Mean:	-	-	0.15
80,000 I. U. vit. A daily + vit. D	♀	84	10	0 07
	♀	68	10	0.00
	♂	81	9	0.03
	Mean:	-	-	0.03
Rachitic diet				
Average of 2 rats	-	83	12	0.88

Table 49 (cont.)

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	mg ascorbic acid per 100 ml serum
Basal diet + excess of vit. A + vit. C				
25,000 I.U. vit. A daily	♀	80	40	0.64
	♂	96	40	0.87
	♀	84	40	0.74
	♂	68	40	0.63
	♂	81	40	1.20
	Mean:	-	-	0.82
50,000 I.U. vit. A daily	♀	40	29	1.65
	♀	41	35	0.56
	♂	51	38	1.05
	♀	43	38	1.60
	♂	48	20	1.07
	♀	49	20	1.14
	♀	45	20	0.63
	♀	53	21	0.70
	♂	40	21	0.91
	♂	41	21	0.93
	Mean:	-	-	1.02
52,500 I.U. vit. A daily	♀	64	21	1.13
60,000 I.U. vit. A daily	♀	95	28	0.73

of vitamin A in "subtoxic" doses caused no reduction of the vitamin C level in the blood, in which case practically no symptoms except a moderate reduction in the weight gain were observed.

Although there is no direct relation between the magnitude of the overdosage with vitamin A and the reduction of the vitamin C level in the blood, the average vitamin C content of the blood was below normal in all groups given toxic doses of vitamin A. In some cases it was reduced to zero.

While the average vitamin C content of the serum in rachitic rats given no excess of vitamin A was normal, it was very low in rats given excess of vitamin A in addition to the rachitic diet. Simultaneously this combination gave rise to pronounced symptoms of hypervitaminosis A.

When the vitamin C was given in addition to the excess of vitamin A, the blood level was in all cases normal or above normal, 24 hours after the last dose of vitamin C.

The excreted amount of vitamin C in the urine per rat in 24 hours was less in hypervitaminotic rats than in normal control animals (see table 50). Simultaneously it was found that the excreted amount of urine in 24 hours was usually only ⅔ of the normal. It should be noted, however, that the urine was collected during 24 hours periods, and that in control experiments, more than 50 % of the ascorbic acid content was destroyed in the urine when left standing at room temperature during 24 hours.

Table 50.

Average Excretion of Ascorbic Acid in Urine in Hypervitaminotic Rats, Compared with Normal Rats.

Conditions of experiment	Daily dose of vit. A, I. U.	mg ascorbic acid excreted per rat in 24 hours
Basal diet (control)	20	0.1
Basal diet + excess of vit. A	10,000	0.0
	37,000	0.0
	50,000	0.0
	60,000	0.0
	50,000— 90,000	0.0
	100,000	0.0
Basal diet + excess of vit. A + 50 mg ascorbic acid by subcutaneous injection	50,000—60,000	8.0—24.0

This may probably explain the low ascorbic acid values found in the urine both from hypervitaminotic and normal rats in the present experiments.

For the purpose of examining the vitamin C content of the serum at various stages of the experiment in rats given excess of vitamin A, the following experiment was carried out:

Experiment 30.

10 adult rats with initial body weights between 146 and 179.5 grams were given 100,000 I. U. vit. A daily in the form of whale liver oil concentrate. Half of this dose was given mixed in the basal diet, and the other half by dropping pipet.

The rats were killed and examined at 4 day intervals, and the hemoglobin and ascorbic acid contents of the serum were determined by the technique described on page 22. Two rats were examined each time, the last two rats being examined on the 20th day of the experiment. The results are given in tables 51 and 52.

On this dose, loss of weight was observed in all rats, except for the two rats which were killed first, — on the 4th day of the experiment.

In the first two animals examined at the end of 4 days, there were no clinical symptoms, and apart from swollen visceral lymph glands, no pathological findings were made at autopsy. In the remaining rats, alopecia and soreness around the mouth and eyes were observed from the 8th day. In one case both hind legs appeared paralysed at the end of 16 days, and at autopsy this was found to be probably caused by large muscular hemorrhages. By postmortem examination swollen visceral lymph glands were observed in most cases. In all cases the blood coagulated within 5 minutes, and the serum colour was 7 in two cases.

From table 51 it appears that a decrease of the ascorbic acid content of the serum takes place in rats given toxic excess of vitamin A as the symptoms of hypervitaminosis A develop. Thus the ascorbic acid content of the serum was considerably less at the end of 20 days, when a marked

Table 51.

Ascorbic Acid Content of the Serum at Various Stages of the Experiment in Rats Receiving Excess of Vitamin A.

No. of days from beginning of experiment until collection of sample	mg ascorbic acid per 100 ml serum		
	Rat 1	Rat 2	Average
4 days	0.765	0.750	0.758
8 „	0.410	0.300	0.355
12 „	0.725	0.175	0.450
16 „	0.430	0.280	0.355
20 „	0.070	0.360	0.215

loss of weight had occurred, than on the 4th day of the experiment, when the rats where still unaffected.

The hemoglobin showed practically normal values in all cases, as is evident from table 52.

Table 52.

Hemoglobin in Rats Receiving Excess of Vitamin A, at Various Stages of the Experiment.

No. of days from beginning of experiment until collection of sample	Hb %		
	Rat 1	Rat 2	Average
4 days	103	107	105
8 „	100	98	99
12 „	95	87	91
16 „	96	86	91
20 „	93	95	94

The ash and mineral contents of the bones in rats in this experiment are given in table 53. From this table it appears that no abnormalities could be detected in the ash or mineral content of the bones.

Table 53.

Ash and Mineral Contents of Femurs in Rats of the Same Age Receiving Excess of Vitamin A, at Various Stages of the Experiment.

Duration of experiment, days	Ash, in % of dry bone			Ca, in % of ash			P, in % of ash		
	Rat 1	Rat 2	Average	Rat 1	Rat 2	Average	Rat 1	Rat 2	Average
4	53.7	52.6	53.1	40.0	32.3	36.2	18.1	17.6	17.9
12	56.4	60.6	58.5	34.6	-	34.6	15.8	-	15.8
16	-	58.4	58.4	-	36.6	36.6	-	17.5	17.5
20	56.8	-	56.8	38.6	-	38.6	16.8	-	16.8

b) The Effect of Vitamin C in Hypervitaminosis A.

In the following experiments, the effect of various doses of vitamin C given by subcutaneous injection or mixed in the basal diet, was studied in rats given various doses of vitamin A. In some experiments the ascorbic acid was injected subcutaneously to eliminate the possibility of deterioration of the ascorbic acid exposed to the air in the food mixture, — or of incomplete absorption, or destruction in the intestinal tract.

Examination of the ascorbic acid level of the blood in rats given 50 mg ascorbic acid mixed in the diet, indicated, however, that the ascorbic acid was amply absorbed when mixed in the basal diet, and only very slight deterioration of the ascorbic acid content of the food mixture was found when it was left standing 24 hours in room temperature, as judged by the indophenol method.

For the purpose of preliminary investigations, one rat with an initial body weight of 73 grams, which for the purpose of another investigation was given 50,000 I. U. vit. A in the form of whale liver oil daily, and which showed distinct symptoms of hypervitaminosis A with limping, fractures, soreness, scruffiness, changes in the pelt, weakness and eye symptoms, was from the 40th day given 50 mg ascorbic acid daily by subcutaneous injection, in addition to the unchanged doses of vitamin A. Shortly after this marked improvement in the general condition occurred, the rat appearing quite normal within two days after the commencement of this treatment. At the end of 70 days, however, a marked loss of weight was observed, and death followed suddenly on the 78th day, although the rat appeared quite normal and free of symptoms by clinical examination the previous day. At autopsy large hemorrhages were found in both adrenals, which were particularly large on the left side, the entire kidney being surrounded by coagulated blood.

In a rachitic rat, which for the purpose of another experiment had been given excess of vitamin A (50,000 I. U. vit. A daily — 625 I. U./g body weight), as well as vitamin D in curative doses in addition to the rachitic diet during a period of 20 days, distinct symptoms of hypervitaminosis A with weakness, eye symptoms, alopecia and fractures were detected at the end of this period. From the 20th day, 50 mg ascorbic acid was given daily by subcutaneous injection in addition to the excess of vitamin A, the rachitic diet and vitamin D, for a further period of 50 days.

Two days after the first dose of vitamin C a marked improvement of the general condition was observed, the rat appearing stronger and healthier. From the third day the rat was free of any clinical symptoms, — the pelt was normal, there were no eye symptoms, and no further fractures occurred. The weight, however, remained quite level throughout the experiment. In rats which were kept under identical conditions with the exception of the additional supply of vitamin C, the symptoms progressed.

No ascorbic acid was given during the last 5 days before the rat was killed. At the conclusion of the experiment there were still no clinical symptoms, no alopecia, no soreness or hemorrhages.

At autopsy, the liver was fatty on the cut surface, and the adrenals appeared small and atrophic. Swollen visceral lymph glands were observed, but no internal hemorrhages.

The hemoglobin was 93 % at the end of 20 days, and 79 % at the conclusion of the experiment, when no ascorbic acid had been given for a period of 5 days, — at which time the ascorbic acid content of the serum was 0.175 mg per 100 ml serum.

Experiment 31.

The purposes of this experiment was to examine whether additional supply of vitamin C had any influence on any of the symptoms of hypervitaminosis A in rats, when the excess of vitamin A was given in doses which otherwise invariably gave rise to symptoms of hypervitaminosis A in rats of this age.

A group of 5 young rats with initial body weights between 68 and 96 grams was given 25,000 I. U. vit. A daily in the form of whale liver oil given by catheter, in addition to the adequate basal diet. This dose corresponded to approximately 300 I. U. vit. A/g body weight daily at the commencement of the experiment. In addition to this 50 mg ascorbic acid per rat per day was given continuously throughout the experiment. Prior to the commencement of the experiment, the experimental rats received 50 mg ascorbic acid per rat daily mixed in the basal diet during a period of 6 days.

Another group of 5 young rats with initial body weights between 74 and 102.5 grams was used as control and was given the adequate basal diet only. In both groups the weight curve was recorded during a period of 6 days before the commencement of the experiment. The average weight graphs are given on page 112.

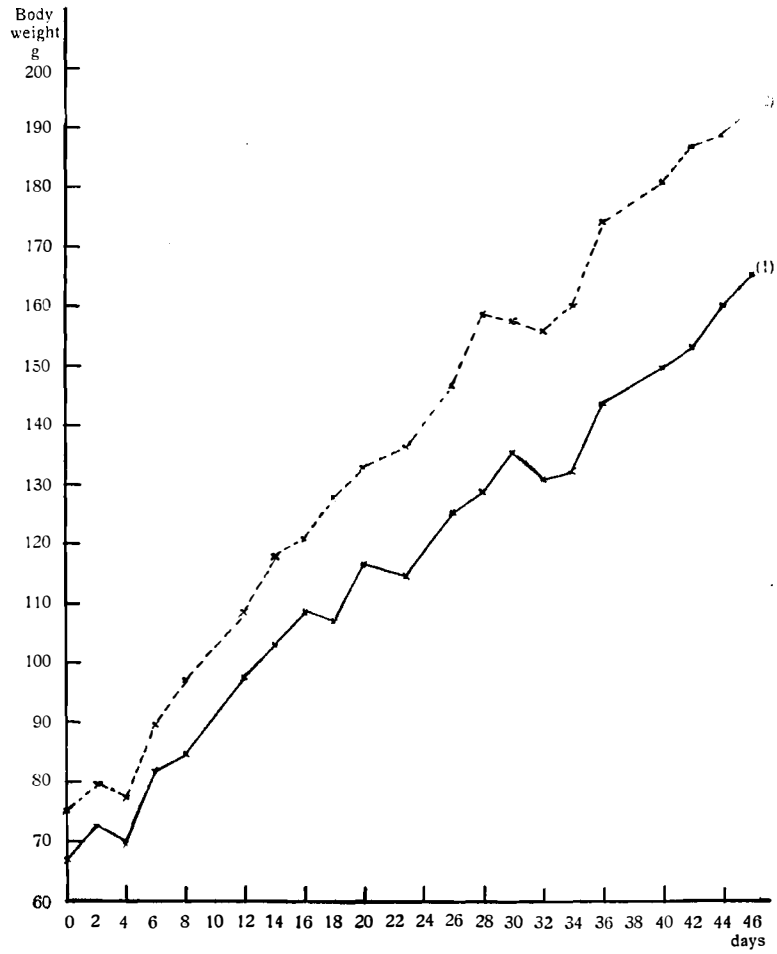
Throughout the experiment, which lasted 41 days, the rats in both groups remained normal and free of any symptoms.

At the conclusion of the experiment, one of the rats in the group receiving excess of vitamin A accidentally fell 1½ meters, without incurring any fracture or the slightest injury. This may be taken as evidence of the fact that the rat suffered from no tendency to spontaneous fractures. In agreement with this, X-ray examination of the long bones showed normal conditions.

At the end of the experiment, the rats were killed and examined in the usual manner after being fasted for 24 hours. They were all free of symptoms and were in good condition. There were normal amounts of subcutaneous and visceral fat. The pelt was normal and the skeletal system was well developed. At autopsy normal organs were found. In one of the rats the liver, kidney, adrenal, and pancreas were examined histologically. Hyperemia was observed in the kidney and pancreas, otherwise no definite pathological findings were made in the organs stained by hematoxylin-eosin. In the liver stained by sudan IIII, dense deposits of sudanophil droplets were seen throughout the organ, both in and between the liver cells. In the adrenal only slight deposits of sudanophil droplets were seen in the cortex, except for a band of dense deposits in the zona glomerulosa.

The results of various laboratory examinations are given in table 54. The hemoglobin was normal, being 104—112 %, as against 81—102 % in the control group. The average vitamin A content of the serum was 21.5 I. U. vit. A, 24 hours after the last dose of vitamin A, and the ascorbic acid content of the serum was from 0.63 to 1.20 mg per 100 ml, 24 hours after the last dose of vitamin C. The average ascorbic acid content of the liver was 9 mg/100 g, and of the adrenals 164 mg/100 g. The weight of the left adrenal was in two cases: 0.0269 and 0.0241 g respectively, — corresponding to 0.012 and 0.016 % of the body weight.

From table 54 it will be seen that the vitamin A content of the serum 24 hours after the last dose of vitamin A, as well as the vitamin A content of the liver, was very high in rats given excess of vitamin A (25,000 I. U. daily), in addition to 50 mg vitamin C daily, and was of the same order as in rats given excess of vitamin A without additional supply of vitamin C. Similar gross doses of vitamin A without additional supply of vitamin C given to rats of similar age during the same period of time, invariably produced distinct symptoms of hypervitaminosis A.



Graph No. 11. Average weight graphs for rats given 50 mg ascorbic acid daily in addition to excess of vitamin A (25,000 I. U. vit. A. — or 300 I. U. vit. A/g body weight daily) (1), compared with normal rats (2).

It may therefore be concluded that under these conditions — 50 mg ascorbic acid per rat per day offered full protection against the injurious effect of 25,000 I. U. vit. A daily, except for the absence of normal weight gain.

Experiment 32.

In this experiment 50,000 I. U. vitamin A was given daily to a group of 5 young rats with initial body weights between 40 and 50 grams (approximately 1,180 I. U. vit. A/g body weight at the commencement of the experiment). In addition to this 50 mg ascorbic acid was given by subcutaneous injection daily, and the same amount of vitamin C per rat was daily mixed in the basal diet.

Table 54.

Results of Various Laboratory Examinations in Rats Given Excess of Vitamin A + Vitamin C. (Exp. 31.)

Conditions of the experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Hb %	I.U. vit. A per g serum, 24 hrs after last dose	mg/100 ml asc. acid in serum 24 hrs after last dose. Fasted 24 hrs	mg/100 g asc. acid in liver	mg/100 g asc. acid in adrenals	Weight of left adrenal, g	Weight of left adrenal in % of body weight	I.U. vit. A/g liver
Basal diet +	1	♀	80	40	106	-	0.64		-	-	-	20,000
25,000 I.U. vit.	2	♂	96	40	104		0.87					
A daily (300	3	♀	84	40	104	21.5	0.74	9	164	0.0269	0.012	
I.U./g body	4	♂	68	40	106		0.63			0.0241	0.016	
weight) + vit.C	5	♂	81	40	112		1.20		-	-	-	
	Mean:		-	-	106	21.5	0.82		164	0.0255	0.014	20,000

The vitamin A was given in the form of whale liver oil concentrate (200,000 I. U./g) by dropping pipet. As a source of vitamin C, natrii l-ascorbinas dissolved in aqua sterilisata dest. was used for injection (1 ml of this solution contained the equivalent of 0.1 g ascorbic acid), and simultaneously one ascorbic acid tablet containing 50 mg ascorbic acid was given per rat per day mixed in the basal diet. The ascorbic acid content was controlled by titration against 2, 6-dichlorophenol-indophenol.

The average initial body weight of the rats in this group was 44.6 grams. Apart from absence of weight gain during the first four days of the experiment, the weight gain was only slightly less than normal during the rest of the experiment, and considerably higher than the rats of the same age receiving similar doses of vitamin A without vitamin C, as is illustrated in table 55.

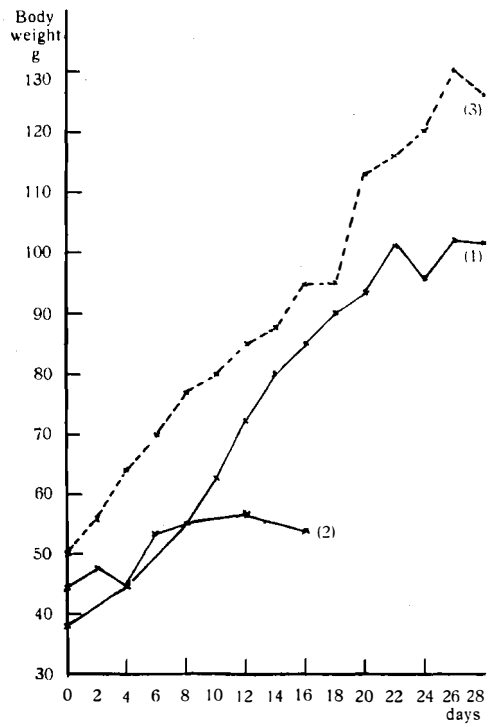
Table 55.

Average Daily Weight Gain in 10-Day Periods in Rats Receiving Excess of Vitamin A With and Without Additional Supply of Vitamin C.

No. of days from beginning of the experiment	0—10 days	10—20 days	20—30 days
Normal	3.0	3.0	2.9
Basal diet + excess of vit. A + vit. C	1.8	3.1	2.2
Basal diet + excess of vit. A	2.0	—0.2	-

The average weight graphs are given on page 114 (graph no. 12).

In the urine, Heller's test and the benzidine test were negative, and by microscopical examination of the centrifuged urine, no red blood cells were seen. 8 mg ascorbic acid were excreted per rat in 24 hours by the urine. The benzidine test in the feces gave negative results.



Graph No. 12. Average weight graph for rats given 100 mg ascorbic acid daily in addition to excess of vitamin A (50,000 I. U. vit. A daily) (1), compared with that of rats given similar doses of vitamin A without vitamin C (2) and normal rats (3).

Each rat was clinically examined daily throughout the experiment. Apart from a short-lasting weakness on the 10th day, and limping on the left fore leg in one of the rats from the 34th day (X-ray examination revealed fractures of the radius and ulna in this rat), the rats remained free of any clinical symptoms throughout the experiment. There were no changes in the pelts, no alopecia, no eye symptoms and no hemorrhages.

One of the rats had exophtalmus of the left eye, which gradually became more pronounced during the experiment. This exophtalmus was found to be caused by a large intraorbital tumour on the optical nerve, which apparently had nothing to do with hypervitaminosis A.

The individual rats in this group were killed and examined at various stages of the experiment. Four of the 5 rats were killed at the end of 30—38 days after the beginning of the experiment. In the remaining rat, the vitamin C was removed at the end of 38 days, and the animal was kept on the ordinary basal diet with addition of the excess of vitamin A in unchanged doses during a period of 37 days. Shortly after the removal of the vitamin C loss of weight was observed, and an increasing weakness occurred from the 49th day, — i. e. 11 days after the removal of vitamin C.

At autopsy, no pathological findings were detected, except for the previously mentioned fracture in one of the rats, and the intraorbital tumour.

In three of the rats, the liver, kidneys, and adrenals were examined histologically. Hyperemia was detected in the liver and kidney. A large number of vacuoles were seen in the liver, but there appeared to be no degeneration of the renal tubules, and no red blood cells outside the capillaries in the internal organs examined. In the liver stained by sudan III, dense deposits of sudanophil droplets were seen in and between the liver cells (Kupffer cells). In the adrenal, moderate

deposits of sudanophil droplets were seen in the zona glomerulosa, and only slight deposits in the rest of the cortex. There was no fatty degeneration of the kidney.

In 4 out of the 5 rats no significant pathological conditions were found by X-ray examination of the long bones, during the period when excess of vitamin C was given in addition to excess of vitamin A. In only one case there was thinning of the bone shafts, and fractures of the left radius and ulna. Following the removal of the excess of vitamin C, however, an increased thinning of the bone shafts and the cortical shadow was observed, and at the conclusion of the experiment, distinct deformities of the compact bones were observed in this rat.

The results of various laboratory examinations are given in table 56.

Table 56.

Results of Blood Examinations in Rats Given Excess of Vitamin A + Vitamin C.

Conditions of experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Time killed after last injection of vitamin C	Hb %	Red blood cells, millions per l/1000 ml	Colour index	Ascorbic acid in serum, mg/100 ml	Serum colour (Meulengrachts)	Prothrombin time, seconds
Excess of vitamin A (50,000 I.U. daily) + excess of vitamin C (50 mg mixed in diet + 50 mg by subcutaneous injection).	1	♀	40.0	29	1 hour	102	5.1	1.0	1.65	-	-
	2	♀	41.5	35	24 hrs	108	-	-	0 56	-	-
	3	♂	51.7	38	24 hrs	99	-	-	1.05	3	-
	4	♀	46.8	75 [1]	37 days	74	4.6	0.8	0.13	12	26.5
	5	♀	43.1	38	1 day	102	-	-	1.60	-	-
	Mean:		-	-	-	97	4.9	0.9	1.00	8	-

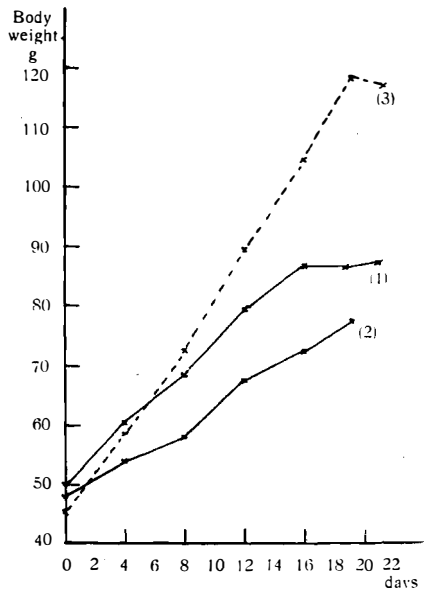
[1] Vitamin C removed at the end of 38 days.

Table 57.

Ash and Mineral Contents of Femurs in Rats Receiving Excess of Vitamin A (50,000 I. U./Day) + Vitamin C (100 mg/Day).

Rat no.	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
1	40.0	29	47.1	37.6	19.7	24	2.0	0
2	41.5	35	53.8	33.7	17.8	23	2.0	+
3	51.7	38	52.7	31.5	16.8	29	2.3	0
4	46.8	75 [1]	58.9	36.5	17.9	27	2.0	0
5	43.1	38	51.4	31.9	18.3	25	2.0	0
Mean:	-	-	52.8	34.2	18.1	27	2.1	-

[1] Vitamin C removed at the end of 38 days.



Graph No. 13. Average weight graphs for rats given different doses of vit. C: 50 mg daily (1), and 100 mg daily (2) in addition to excess of vitamin A (50,000 I. U. daily), compared with normal rats (3).

No abnormality was detected in the ash and mineral contents of the bones (see table 57).

In a group of 5 young rats with similar, or slightly lower initial body weights (see experiment 16, p. 55) receiving similar doses of vitamin A without additional supply of vitamin C, distinct symptoms of hypervitaminosis A were detected in all rats within 10 days. They all suffered from marked weakness, alopecia, scruffiness, as well as multiple fractures and the general condition of these rats was very poor. In one of them this dose of vitamin A proved lethal at the end of 13 days, and in the remaining 4 at the end of 18 days. X-ray examination of the long bones and postmortem examination revealed typical hypervitaminosis A changes in all cases.

From this experiment it appears evident that large doses of vitamin C given by subcutaneous injection proved beneficial to rats given excess of vitamin A.

Experiment 33.

In this experiment three groups of young rats of the same age with initial body weights of approximately 50 grams were used.

One group of three rats was used as control and received the ordinary basal diet only.

A second group of 5 animals was given 50,000 I. U. vitamin A daily (approximately 1,000 I. U./g body weight) by dropping pipet, in the form of whale liver oil concentrate, in addition to the basal diet. In addition to this 50 mg ascorbic acid was given per rat per day mixed in the basal diet, and 50 mg ascorbic acid per rat daily by subcutaneous injection.

A third group of 5 rats received the same dose of vitamin A and 50 mg ascorbic acid per rat per day, mixed in the basal diet.

The control rats remained normal throughout the experiment, which lasted during a period of approximately 20 days.

In the second group, the weight gain was less than normal (see graph no. 13, p. 116), although two of the rats remained free of any clinical symptoms through-

out the experiment. In one rat slight soreness and alopecia around the eyes was observed; in the remaining two rats, swelling of the palpebrae occurred. Apart from this no symptoms were observed by clinical examination in this group.

X-ray examination at the conclusion of the experiment showed bone changes typical for hypervitaminosis A, — such as abnormally thin bone shafts and thinning of the cortical shadow, in all rats in this group. In two cases fractures of the fibula with marked callus formation were observed, although previous clinical examination had failed to detect any limping or fractures.

The rats were killed and examined at the end of 20 days. The last dose of vitamin C had been given 24 hours previously. Two of the rats were given the last dose of vitamin A, 24 hours before being killed, — and the remaining three rats, two hours before being killed, for the purpose of examining the vitamin C level in the blood under these conditions.

The vitamin C content of the serum was considerably less in the rats which had been given 50,000 I. U. vit. A 1—2 hours before the blood sample had been collected, than in the rats which had received no vitamin A for the last 24 hours.

By postmortem examination no pathological findings were made apart from the slight eye symptoms already mentioned, and the swollen visceral lymph glands in one case. There were no hemorrhages and no fractures.

In two of the rats the liver, kidneys, adrenals, spleen, pancreas, and in one rat also the testes and bones were examined histologically. Hyperemia was detected in the kidney in one case, and irregularity of bone structure with fracture and hemorrhages in the tibia. Otherwise no pathological findings were detected in the adrenal, kidney, pancreas, spleen, or testes when stained by hematoxylin-eosin. In the liver stained by sudan III slight deposits of sudanophil droplets were seen. In the adrenal moderate sudanophil deposits were seen in the periphery of the cortex and on the border of the medulla and cortex.

The results of various laboratory examinations are given in table 58.

In the third group, which received 50,000 I. U. vit. A daily and 50 mg ascorbic acid per rat per day mixed in the basal diet, fractures occurred in two of the 5 rats. In two cases there was slight alopecia around the mouth, in one case slight swelling of the palpebrae, and in one case slight soreness around the mouth, otherwise no clinical symptoms were observed.

In one of the rats there were fractures of both radius and ulna, and in the other case there was fracture of the fibula with marked callus formation. Apart from this, roentgenograms showed that the bone shafts were abnormally thin.

The rats were killed and examined at the end of 21 days. Three hours before they were killed, 50 mg ascorbic acid per rat was mixed in the basal diet and they all received 50,000 I. U. vit. A, one hour before being killed.

At autopsy no pathological findings were made except for the already mentioned fractures, marked hyperemia in one case and hemorrhage in the small intestines in another case.

In one of the rats the liver, kidneys, adrenals, pancreas, and thyroid gland were examined histologically. Hyperemia and erythrocytes in some of the renal tubules and the spaces of Bowman's capsules were observed. Otherwise no significant pathologico-anatomical findings were detected in the internal organs examined.

The control group was killed and examined at the end of 23 days, and by postmortem examination normal organs were found.

From table 58, showing the results of various laboratory investigations in this experiment, it will be seen that in most cases the hemoglobin was normal. There was a slight anemia in two cases, but in none of these cases hemorrhages were observed which could explain the anemia as a bleeding anemia. The prothrombin

time was normal in the one case examined, as was the phosphorus content of the serum.

The ash and mineral contents of the bones are given in table 59, showing no marked difference between these rats and normal rats of the same age.

From this experiment it may be concluded that massive doses of vitamin C (50—100 mg per rat per day) did not prevent all the symptoms of hypervitaminosis A in young rats, when given massive doses of vitamin A (1,000 I. U./g), such as bone abnormalities, reduced weight gain, soreness and alopecia around the mouth and eyes. The general condition of the rats appeared less affected, however, and the incidence of hemorrhage was less in these rats than in rats given similar doses of vitamin A without vitamin C.

Table 58.

Results of Various Laboratory Examinations in Rats Given Excess of Vitamin A + Vitamin C (Exp. 33).

Conditions of the experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	mg asc. acid in 100 ml serum	Prothrombin time, seconds	Serum iron, γ %	P in serum, mg/100 ml
Basal diet (control)	1	-	45	23	95	-	-	-	-	302	-
	2	-	45	23	100	-	-	-	15½	-	-
	3	-	47	23	97	-	-	-	-	349	-
	Mean:		-	-	97	-	-	-	-	321	-
Basal diet + excess of vit. A (50,000 I. U. daily) + vit. C (100 mg daily)	1	♂	48	20	82	4.8	0.9	1.07 [2 1]	-	-	-
	2	♀	49	20	86	-	-	1.14 [2 1]	-	-	-
	3	♀	46	20	80	-	-	-	-	220	-
	4	♀	50	20	90	-	-	-	15	-	-
	5	♀	45	20	87	4.8	0.9	0.63 [3 1]	-	-	-
	Mean:		-	-	85	4.8	0.9	0.95	-	-	-
Basal diet + excess of vit. A (50,000 I. U. daily) + vit. C (50 mg daily)	1	♀	53	21	90	-	-	0.70 [4]	-	-	-
	2	♀	59	21	86	-	-	-	-	-	-
	3	♂	59	21	76	-	-	-	-	-	5.9
	4	♂	40	21	87	-	-	0.91 [4]	-	-	-
	5	♂	41	21	73	-	-	0.93 [4]	-	-	-
	Mean:		-	-	82	-	-	0.85	-	-	-

[1] Sample collected 24 hours after last dose of vitamin C.
[2] Sample collected 24 hours after last dose of vitamin A.
[3] Sample collected 1—2 hours after last dose of vitamin A.
[4] 50 mg asc. acid mixed in diet 3 hours before killed.

Table 59.

Ash and Mineral Contents of the Femurs in Rats Given Excess of Vitamin A + Vitamin C. (Exp. 33.)

Conditions of the experiment	Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
Basal diet + excess of vit. A (50,000 I. U./day) + vit. C (100 mg/day)	1	♂	48	20	52.0	31.4	19.0	24	2.0	+
	2	♀	49	20	51.0	27.6	20.3	23	2.0	0
	3	♀	46	20	50.2	29.3	18.1	22	2.0	0
	4	♀	50	20	48.0	33.4	21.4	21	1.9	0
	5	♀	45	20	52.9	29.2	20.0	22	2.0	+
	Mean:		-	-	50.8	30.1	19.7	22	2.0	-
Basal diet + excess of vit. A (50,000 I. U./day) + vit. C (50 mg/day)	1	♀	53	21	51.1	26.2	16.0	24	1.9	+
	2	♀	59	21	55.8	26.6	18.9	23	2.0	+
	Mean:		-	-	53.5	26.4	17.5	24	2.0	-

Experiment 34.

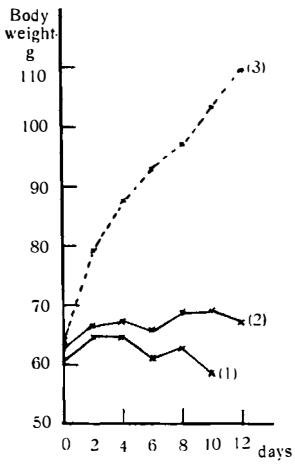
The purpose of this experiment was to examine the effect of large doses of vitamin C in rats given excess of vitamin A acetate.

In this experiment a group of 4 rats with initial body weights between 62 and 64 grams was given 50 mg ascarbic acid mixed in the basal diet, and 50 mg ascorbic acid by subcutaneous injection per rat per day in addition to vitamin A acetate, in amounts corresponding to 52,500 I. U. vit. A per rat daily. The vitamin A acetate was dissolved in a minimum of peanut oil, and part of the vitamin A dose was given mixed in the diet, and part by dropping pipet.

This group was compared with a group of three rats which had the same body weight, and which were given the same dose of vitamin A acetate without vitamin C (see experiment 2, page 26) and a control group given basal diet only. The average weight graphs in these three groups are given on p. 120 (graph no. 14).

In the group receiving large doses of vitamin C in addition to the excess of vitamin A, three of the rats died at the end of 13 days, and one was killed when moribund on the 21st day. Fractures were observed in all cases, eye symptoms and scruffiness in two, and weakness and alopecia in one case. By postmortem examination in one case, no pathological findings were detected in the internal organs. In two cases the lungs were fiery red, and in one case the pancreas appeared enlarged.

Histologically, hyperemia was detected in the internal organs, such as the liver, kidneys, adrenals and pancreas from the two rats examined. In the kidney there was slight degeneration of the tubules, some of which contained free blood in one case. In the liver a large number of vacuoles were seen in one case. When stained by sudan III, dense deposits of sudanophil droplets were seen between the liver cells, while only a few droplets were detected in the actual liver cells. In the adrenal very few sudanophil droplets were seen in the cortex. No fatty degeneration was observed in the kidney.



Graph No. 14. Average weight graphs for rats given excess of vitamin A acetate, 52,500 I. U. vitamin A daily (1), and rats given the same dose of vitamin A acetate in addition to 100 mg ascorbic acid daily (2), compared with normal rats (3).

The results of various laboratory examinations are given in table 60 (see also table 2).

In the group which received the same dose of vitamin A acetate without vitamin C, the mentioned dose of vitamin A proved lethal in two rats at the end of 11 days, and in one rat at the end of 21 days. In all cases distinct symptoms of hypervitaminosis A were observed with fractures, changes in the pelts and eye symptoms.

From this experiment it may be concluded that large doses of vitamin C offered no protection against 52,500 I. U. vit. A daily, when given both mixed in the diet and by dropping pipet.

Table 60.

Results of Various Laboratory Examinations in Rats Receiving Excess of Vitamin A Acetate (52,500 I.U. Daily) and Vitamin C (100 mg Daily). (Exp. 34.)

Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Asc. acid in serum, mg/100 ml	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
1	♀	62	13	-	-	-	-	-	-	-	-
2	♀	64	21	84	1.13	53.0	43.5	19.0	23	1.5	+
3	♂	62	13	-	-	-	-	-	-	-	-
4	♀	62	13	-	-	53.1	37.4	24.9	-	-	+
	Mean:	-	-	-	-	53.1	40.4	21.9	-	-	-

Experiment 35.

In a further experiment the effect of large doses of vitamin C was studied in rats receiving larger doses of vitamin A, in the form of highly concentrated and purified sources of vitamin A, produced from shark liver oil (640,000 I. U./g).

Four drops of this oil, corresponding to 60,000 I. U. vit. A were given daily by dropping pipet to a group of 4 rats with initial body weights between 68.5 and 95 grams, in addition to the usual basal diet, and 50 mg ascorbic acid per rat by subcutaneous injection.

This group was compared with a group of 4 rats in a previous experiment (experiment 3, page 27), which had the same initial body weight and which received identical doses of the same vitamin A oil without vitamin C.

In the latter group, given excess of vitamin A only, distinct symptoms of hypervitaminosis A were detected in all cases, such as changes in the pelts, with scruffiness and alopecia, eye symptoms and muscular weakness, and fractures were found in three of the 4 rats. At autopsy subcutaneous hemorrhages were detected in one of the rats, and abscesses in the kidney in three cases.

In the group receiving 50 mg ascorbic acid daily in addition to excess of vitamin A, the rats were affected in the same way as the previous group, although the general condition in some of them appeared less affected. One rat died at the end of 9 days, and one at the end of 41 days. The remaining two were killed and examined at the end of 28 and 42 days. In all cases scruffiness, soreness around the eyes, as well as limping was observed. In three cases fractures occurred and in one case swelling of the palpebrae was observed. In one of the rats a marked improvement of the symptoms occurred towards the conclusion of the experiment.

A urine sample was collected during a 24 hour period from the 10th day of the experiment, the excreted amount of ascorbic acid per rat in 24 hours being 24.5 mg.

There was marked fragility of the bones. In two cases fractures occurred as a result of the rats pressing their hind legs against the hand during dosage.

By postmortem examination, no pathological findings were detected in the internal organs in two cases. In one case there was marked hyperemia and scattered hemorrhages, as well as enlarged adrenals, and in one case the lungs were fiery red. The cut surface of the liver appeared fatty in two cases.

Two of the rats were examined histologically. In the liver stained by sudan III, large deposits of sudanophil droplets were seen between the liver cells (Kupffer cells), while practically no sudanophil deposits were detected in the actual liver cells. In the adrenal, dense sudanophil deposits were seen in the zona glomerulosa of the cortex. In the pancreas and spleen no significant pathological findings were detected.

X-ray examination of the long bones showed, except for the cases where fractures occurred, less pronounced changes than in the previous group.

The results of various laboratory examinations are given in tables 61 and 62 (see also table no. 2).

Summary of Results for Chapter 15. The average ascorbic acid content of the liver and of the adrenals appeared to be lower in rats given toxic doses of vitamin A, than in normal control animals (see table 48). The average ascorbic acid content of the serum was also lower in hypervitaminotic rats than in normal rats, or in rats given the liver oil freed of its vitamin A content. Moderate excess of vitamin A in sub-toxic doses, however, caused no reduction of the vitamin C level in the blood (see table 49). A decrease of the ascorbic acid content of the serum occurred in the course of the experiment in rats given toxic doses of vitamin A, as the hypervitaminotic symptoms developed (see table 50).

Table 61.

Results of Blood Examinations in Rats given Excess of Vitamin A Concentrate (60,000 I. U. Vit. A Daily) with Addition of Vitamin C (Experiment 35.)

Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	Prothrombin time, seconds	Asc. acid in serum, mg/100 ml	Serum Colour	Excreted asc. acid in urine mg/r/24 hrs.
1	♀	68.5	9	-	-	-	-	-	-	
2	♀	95.0	28	80	4.5	0.9	-	0.73	15	
3	♀	89.0	42	74	3.4	1.0	17	-	-	2.5
4	♀	86.0	41	-	-	-	-	-	-	
Mean:	-	-	-	77	3.9	1.0	-	-	-	2.5

Table 62.

Ash and Mineral Contents of the Femurs in Rats Given Excess of Vitamin A Concentrate (60,000 I. U. Daily) with Addition of Vitamin C. (Experiment 35.)

Rat no.	Sex	Initial body weight, g	Duration of experiment, days	Ash, % of dry bone	Ca, % of ash	P, % of ash	Length of femur, mm	Width of femur, mm	Fractures
3	♀	89	42	58.6	28.9	17.6	28	2.0	0
4	♀	86	41	53.1	29.5	18.1	25	2.0	+
Mean:		-	-	55.9	29.3	17.9	27	2.0	-

The excreted amount of ascorbic acid in the urine per rat in 24 hours was less in hypervitaminotic rats than in normal control rats (see table 50).

Subcutaneous injection of ascorbic acid proved beneficial to rats receiving moderate excess of vitamin A. Thus in one experiment, 50 mg ascorbic acid daily offered protection against the injurious effect of 25,000 I. U. vit. A daily (300 I. U. vit. A/g body weight), in rats with initial body weights between 68 and 96 grams. The rats remained normal and free of any symptoms throughout the experiment, which lasted 41 days, except for the absence of normal weight gain. In a second experiment, 100 mg ascorbic acid daily proved beneficial to rats given 50,000 I. U. vit. A daily (1,180 I. U. vit. A/g body weight), while similar doses of vitamin A without additional supply of vitamin C given to a group of rats of similar age caused pronounced symptoms of hypervitaminosis A.

Additional supply of vitamin C did not prevent the development of symptoms of hypervitaminosis A, however, in rats given daily doses over 50,000 I. U. vit. A (see table 63).

Table 63.

Clinical Symptoms in Rats Given Excess of Vitamin A, With and Without Additional Supply of Vitamin C.

Experiment no.	Conditions of experiments	Rat no.	Initial body weight, g	Duration of experiment, days	Lethal result	Reduced weight gain	Weakness	Scruffiness	Alopecia	Soreness of skin	Swelling of palpebræ	Limping	Fractures	Pathological X-ray findings
31	Basal diet (control)	1	90	45	0	0	0	0	0	0	0	0	0	0
		2	103	45	0	0	0	0	0	0	0	0	0	0
		3	95	45	0	0	0	0	0	0	0	0	0	0
		4	88	45	0	0	0	0	0	0	0	0	0	0
		5	74	45	0	0	0	0	0	0	0	0	0	0
	Basal diet + excess of vit.A (25,000 I.U. daily) + vit.C (50 mg daily)	1	80	40	0	+	0	0	0	0	0	0	0	0
		2	96	40	0	+	0	0	0	0	0	0	0	0
		3	84	40	0	+	0	0	0	0	0	0	0	0
		4	68	40	0	+	0	0	0	0	0	0	0	0
		5	81	40	0	+	0	0	0	0	0	0	0	0
32	Basal diet + excess of vit.A (50,000 I.U. daily) + vit.C (100 mg daily)	1	40	29	0	+	+	0	0	0	0	0	0	0
		2	42	35	0	+	+	0	0	0	0	0	+	+
		3	52	38	0	+	+	0	0	0	0	0	0	+
		4	47	75	0	+	+	0	0	0	0	0	0	0
		5	43	38	0	+	+	0	0	0	0	0	0	0
33	Basal diet (control)	1	45	23	0	0	0	0	0	0	0	0	0	0
		2	45	23	0	0	0	0	0	0	0	0	0	0
		3	47	23	0	0	0	0	0	0	0	0	0	0
	Basal diet + excess of vit.A (50,000 I.U. daily) + vit.C (100 mg daily)	1	48	20	0	+	0	0	0	0	0	0	+	+
		2	49	20	0	+	0	0	0	0	0	0	0	+
		3	46	20	0	+	0	0	+	+	0	0	0	+
		4	50	20	0	+	0	0	0	0	+	0	0	+
		5	45	20	0	+	0	0	0	0	+	0	+	+
	Basal diet + excess of vit.A (50,000 I.U. daily) + vit.C (50 mg daily)	1	53	21	0	+	0	0	+	0	0	0	0	+
		2	59	21	0	+	0	0	0	0	+	0	+	+
		3	59	21	0	+	0	0	0	+	0	+	0	+
		4	40	21	0	+	0	0	0	0	0	0	0	+
		5	41	21	0	+	0	0	+	0	0	0	+	+
34	Basal diet (control)	1	64	24	0	0	0	0	0	0	0	0	0	-
		2	63	24	0	0	0	0	0	0	0	0	0	-
	Basal diet + excess of vit.A (52,500 I.U. daily) + vit.C (100 mg daily)	1	62	13	+	+	0	0	0	0	0	+	+	-
		2	64	21	+	+	+	+	+	+	+	+	+	+
		3	62	13	+	+	0	+	0	+	+	+	+	-
		4	62	13	+	+	0	0	0	0	0	+	+	-
35	Basal diet (control)	1	87	28	0	0	0	0	0	0	0	0	0	0
		2	93	33	0	0	0	0	0	0	0	0	0	0
	Basal diet + excess of vit.A (60,000 I.U. daily) + vit.C (50 mg daily)	1	69	9	+	+	0	+	0	+	0	+	0	+
		2	95	28	0	+	0	+	0	+	+	+	+	+
		3	89	42	0	+	0	+	0	+	0	+	0	+
		4	86	41	+	+	0	+	+	+	0	+	+	+

16. *The Adrenals in Hypervitaminotic Rats.*

In this chapter the various observations concerning the adrenals in hypervitaminotic rats will be summarized.

From the previously described experiments it is evident that several abnormalities were detected in the adrenals of hypervitaminotic rats by postmortem examination. In a number of cases they appeared enlarged, a high frequency of accessory adrenals was observed, and increased deposits of pigment were found in a great many cases around the

Table 64.

Weight of Left Adrenal in Young Hypervitaminotic Rats, Calculated in per cent of Body Weight.

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Weight of left adrenal in % of body weight
Basal diet (control)	♀	29.0	69	0.019
	♂	24.0	69	0.018
	♀	26.0	69	0.016
	♀	26.0	69	0.018
	♀	21.0	69	0.016
	♂	68.0	33	0.013
	Mean:	-	-	0.017
Basal diet + excess of vit. A 10,000 I. U. vit. A daily	♂	78.0	16	0.015
	♂	84.5	13	0.008
	♂	68.0	9	0.016
	♂	95.5	19	0.008
	♂	87.0	19	0.010
	Mean:	-	-	0.011
50,000 I. U. vit. A daily	♂	68.0	16	0.043
60,000 I. U. vit. A daily	♂	80.0	16	0.024
	♂	98.5	15	0.018
	♂	68 0	13	0.031
	♂	69.0	9	0.025
	♂	95.0	18	0.014
	Mean:	-	-	0.022
Basal diet + excess of vit. A + vit. B_1 50,000 I. U. vit. A + 1 mg vit. B_1 daily	♂	98.0	33	0.021
	♂	99.0	33	0.030
	♂	68.0	15	0.043
	Mean:		-	0.030
Rachitic diet + excess of vit. A 50,000 I. U. vit. A daily	♀	50.5	14	0.032
Basal diet + excess of vit. A + vit. C 25,000 I. U. vit. A daily	♂	75.0	44	0.012
	♀	69.0	44	0.016
	Mean:	-	-	0.014

Table 65.

Weight of Left Adrenal in Adult Hypervitaminotic Rats, Calculated in per cent of Body Weight.

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Weight of left adrenal in % of body weight
Basal diet (control)	♂	233.0	50	0.008
	♂	169.0	50	0.010
	Mean:	-	-	0.009
Basal diet + excess of vit. A				
25,000 I.U. vit. A daily	♀	160.0	18	0.035
50,000—90,000 I.U. vit. A daily	♂	178.0	16	0.012
	♂	183.0	24	0.015
	♂	214.0	29	0.014
	♂	202.0	44	0.020
	♂	176.0	45	0.013
	♂	220.0	48	0.020
	♂	197.0	13	0.008
	♂	216.0	46	0.015
	♂	203.0	46	0.019
	♂	223.0	46	0.013
	♂	150.0	46	0.020
	Mean:	-	-	0.015
100,000 I.U. vit. A daily	♀	149.0	28	0.027
	♂	163.0	46	0.027
	♂	192.0	46	0.020
	♀	170.0	46	0.029
	♀	161.0	46	0.031
	♀	143.0	46	0.022
	Mean:	-	-	0.026

adrenals, along the aorta, and elsewhere in the internal organs. Histologically, hyperemia was a frequent finding, and in a few cases hemorrhages also occurred in the adrenals. Wolbach and Bessey (1942) have previously reported some atrophy of the zona glomerulosa of the adrenal cortex in hypervitaminosis A.

The weight of the adrenals was recorded in some of the rats. In all cases the left adrenal was weighed and the weight was expressed in per cent of the body weight of the rat. The results are summarized in tables 64 and 65.

From these tables it is evident that the average weight of the adrenals calculated in per cent of the body weight, is higher in rats given massive doses of vitamin A than in normal rats of similar age, in both young and adult animals, which is in agreement with the visual findings by postmortem examination. It is a question as to what extent the observed hyperemia in the adrenals might account for the increase in weight of the gland in hypervitaminotic animals.

Table 66

Approximate Distribution of Sudanophil Deposits in Adrenals in Hypervitaminotic and Normal Rats.

Conditions of experiment	Daily dose of vit. A, I. U.	Sex	Age	Date of examination	Sudanophil deposits in adrenal			
					Medulla	Cortex		
						Z. reticularis	Z. fasciculata	Z. glomerulosa
Basal diet	20	♂	young	29/5	0	+	+	++
	„	♂	„	15/7	-	-	(+)	(+)
	„	♂	„	15/7	0	+	++	++
	„	♂	„	15/7	0	+	++	++(+)
	„	♀	„	15/7	-	+	+++	++
	„	♂♀	adult	6/3	0	(+)	(+)	+
	„	♂	„	15/7	0	+	++	++
	„	♂	„	13/10	-	++	+(+)	+++
	„	♂	„	13/10	0	++	+(+)	+++
	„	♀	„	15/7	-	-	+	+
	„	♀	„	15/7	0	+	+	+
	„	♀	„	15/7	0	+	+	+
	„	♀	„	15/7	0	0	+	+
	„	♀	„	15/7	0	0	(+)	(+)
	„	♀	„	6/9	0	+	+	++
Basal diet + excess of vit. A	10,000	♂	young	30/8	-	+	+++	++++
	„	♂	„	30/8	0	+	++(+)	++++(+)
	„	♀	adult	18/4	0	++++	++++	++++
	„	♀	„	23/4	+	+	+	++++
	„	♂♀	„	23/4	0	+	+	+++
	25,000	♂	adult	16/12	+	+++	++++	++++(+)
	„	♀	„	16/12	+	+++	+++(+)	++++(+)
	30,000	♀	adult	7/12	(+)	++	+	+++++
	37,000	♀	young	6/4	-	++	++	+++
	„	♀	„	16/4	0	++	+++	++++
	„	♂	adult	10/4	+	++++	++++	+++++
	„	♂	„	10/4	-	+++	+++	++++
	45,000	♂	young	15/12	0	+	+	+++
	„	♂	„	20/12	0	+	+	++++
	„	♂	„	20/12	0	+	+	++++
	„	♂	„	20/12	0	+	(+)	+
	„	♂	„	20/12	0	+	+	+++
	50,000	♀	young	6/4	-	+++	+++	+++
	„	♀	„	21/5	0	++	+	+++++
	„	♂♀	„	1/6	-	+(+)	+(+)	+(+)
	„	♂	adult	26/3	+	++	++	++(+)
	„	♀	„	19/5	+	+	+	++
	„	♀	„	19/5	0	+	+	++
	„	♀	„	21/5	0	++++	++++	+++++
	52,500	♀	young	16/5	0	(+)	0	(+)
	„	♂♀	„	16/5	-	-	+	++
	60,000	♀	young	19/5	0	+	+	++
	„	♀	„	29/5	-	++	++	++(+)
	50,000—90,000	♂	adult	2/9	0	+	+	+++
	„	♂	„	9/9	-	+	+++	++++(+)
	„	♂	„	9/9	0	+	++	+++
	„	♂	„	7/10	0	++	+	+++++
	„	♂	„	12/10	0	+	+++	++++
	„	♂	„	12/10	0	++++	++++	+++++
	100,000	♀	young	29/10	+	+	+	++++
	„	♂♀	„	29/10	0	+++++	++++	+++++
	„	♂	adult	29/10	-	+	++	+++(+)

Table 66 (cont.)

Conditions of experiment	Daily dose of vit. A, I.U.	Sex	Age	Date of examination	Sudanophil deposits in adrenal			
					Medulla	Cortex		
						Z. reticularis	Z. fasciculata	Z. glomerulosa
Young from hypervit. mothers	-	♂	young	24/5	-	++++(+)	++++(+)	++++(+)
Large single doses of vit. A	0.7 mill.	♂	young	21/9	0	(+)	(+)	+
	1.6 mill.	♂	adult	21/9	+	+	+	++
Subcut. inj. of excess of vit. A	10,000	♀	young	29/5	(+)	+++(+)	++++(+)	++++(+)
Basal diet + vitamin A-free liver oils:								
bear liver oil	20	♂	adult	18/4	0	(+)	(+)	(+)
	„	♂	„	18/4	0	(+)	(+)	(+)
whale liver oil	20	♂	young	14/5	0	(+)	+(+)	++(+)
	„	♂	adult	6/9	+	++	+++	+++(+)
	„	♀	„	6/9	0	+	++	+++(+)
Basal diet + kitol	20	♀	young	14/4	(+)	+	++	+++
Basal diet + sterols	20	♀	adult	24/5	0	+(+)	+++	++++
Basal diet + excess of vit. A + vit. C	25,000	♀	adult	30/11	+	+	+	++
	50,000	♀	young	10/4	0	+(+)	+	++
	„	♀	„	26/5	0	+	+	+
	„	♂	„	8/6	+	+	+	++++
	„	♀	„	8/6	0	+	++	+++
	„	♂	„	9/6	0	+	+	+++
	52,500	♀	young	18/5	0	(+)	(+)	+
	„	♀	„	26/5	0	(+)	(+)	+
	60,000	♀	young	19/5	0	(+)	(+)	++
Basal diet + excess of vit. A + vit. B_1	50,000	♂	young	1/9	0	+	+++	+++(+)
	„	♂	„	1/9	0	(+)	+	++
Rachitic diet + excess of vit. A	50,000	♂	young	17/3	0	+	++	++(+)
	„	♂	„	21/5	0	+	+	+
	„	♀	„	24/5	0	+	+(+)	++++
	„	♂	„	26/5	0	+	++	+++(+)
	„	♀	„	16/9	0	+	+	++
	„	♀	„	21/9	-	+	+	+++
	„	♂	„	21/9	0	++	+++	+++

In rats given additional supply of vitamin C in addition to excess of vitamin A, there was no increase in the weight of the adrenals in the two cases examined.

From table 66, showing roughly the distribution of sudanophil deposits in the adrenals in hypervitaminotic and normal rats, it is observed that the sudanophil deposits appear to be denser in the adrenal cortex in rats given excess of vitamin A than in normal control rats of the same age and sex, examined during the same period of the year.

The sudanophil deposits were mostly pronounced in the periphery of the cortex, — in zona glomerulosa, but extended generally also into the rest of the cortex (zona fasciculata et reticularis). The droplets were in some cases very large, and entirely filled the space between the cortical cells. In some cases sudanophil droplets were also detected in the adrenal medulla in hypervitaminotic rats. No significant increase in the density of the sudanophil deposits was detected in the adrenal cortex in rats given bear- or whale liver oil, where the vitamin A had been destroyed. The deposits of sudanophil droplets in the adrenal cortex were possibly less pronounced in rats given vitamin C in addition to excess of vitamin A than in rats given excess of vitamin A only.

The cholesterol content of the adrenals was determined by Lumetron's photoelectric colorimeter in two female hypervitaminotic rats, and in two normal rats of the same sex simultaneously, and no significant difference was detected. In the first case the average figure was 1.52 mg (3.8 %), and in the latter case 1.58 mg (4.0 %).

It has previously been mentioned (see page 103, and table 48) that the ascorbic acid content of the adrenals was reduced in hypervitaminotic rats, and that considerable amounts of vitamin A were stored in the adrenals in rats given excess of vitamin A (see page 84, table 32).

17. *The Effect on Rats of the Liver from a Dog given Excess of Vitamin A.*

Experiment 36.

The liver from a hypervitaminotic dog, containing 90,000 I. U. vit. A/g, was given to a group of 5 young rats with initial body weights between 40 and 50 grams. 0.5 grams of this liver, corresponding to approximately 45,000 I. U. vit. A was given to each rat daily, after being ground and mixed in the ordinary adequate basal diet.

One of the rats died at the end of 14 days, one at the end of 15, and the remaining rats were killed and examined at the end of 20 days. One of the rats lost 10 grams during the experiment, and in the remaining rats there was only a very slight weight gain, — the average daily weight increase being approximately 0.6 grams for the whole group.

In all cases soreness around the mouth, nose and eyes, exopthalmus, swelling of the palpebrae and forehead, as well as lack of appetite was observed. Multiple fractures occurred in 4 of the rats, hemorrhages in one, and three of them looked anemic. In one case there was marked alopecia around the anus and elsewhere on the body.

In one of the rats, which died during the experiment, the hind legs, as well as the lower part of the body was completely paralyzed, and there was incontinentia urinae et alvi, although X-ray examination of the spine revealed no fracture, and no hemorrhages were detected in the meninges or in the brain itself. There was marked hyperemia of the brain, however, as well as bleeding from the nose, and large hemorrhages around the fractures. Postmortem examination revealed general hyperemia. The adrenals appeared considerably enlarged and dark in colour. The liver appeared fatty on the cut surface, and the lungs were fiery red.

In the remaining rats no significant pathological findings were revealed by macroscopical postmortem examination, apart from enlarged adrenals. No visceral or subcutaneous hemorrhages were observed apart from hemorrhages around the fractures.

By Best's staining of the liver, glycogen was found to be only very sparingly dispersed in the organ.

The liver and adrenals were examined histologically in all of the rats in this group, and the kidneys, spleen, pancreas, testes and tibia in one of the rats. Marked hyperemia was observed in all cases. There was slight degeneration of some of the renal tubules, some of which were filled with amorphous masses and deposits of calcium. A few red blood cells were seen in some of the spaces of Bowman's capsules. In one of the rats, free blood was seen in the knee joints, and in the tibia periostal and subperiostal hemorrhages were observed, as well as signs of increased bone destruction with destructed bone spicules and large Howship's lacunae, and a large number of osteoclasts. By Sudan III staining of the liver, very dense deposits of sudanophil droplets were seen in, and particularly between, the liver cells. In the adrenal, dense sudanophil deposits were observed in the zona glomerulosa, while only scattered sudanophil droplets were seen in the rest of the cortex.

From this experiment it appears that the liver from a dog which had received excess of vitamin A during a long period, proved equally as toxic to rats as bear liver, bear liver oil (Rodahl, 1949, 1& 2), whale liver oil or purified vitamin A preparations, when given in corresponding doses with regard to the vitamin A content.

Experiment 37.

The glycogen was determined by the technique described by Svensson (1945) in the livers of 15 adult hypervitaminotic rats, which during a period of 14 days had been given approximately 45,000 I. U. vitamin A daily in the form of the same dog liver as used in the previous experiment. The rats, which showed distinct signs of hypervitaminosis A, were fasted 24 hours before they were killed. Simultaneously the glycogen content was determined in the liver of 4 normal adult rats which had also been fasted 24 hours. The results are given in table 67.

From table 67 it is evident that the average glycogen content of the liver is lower in hypervitaminotic rats than in normal rats. In 6 cases it was abnormally low in the first mentioned group (10—20 mg/100 g). This was probably due to inanition.

18. *Miscellaneous Observations.*

In this chapter the results of the various blood examinations in hypervitaminotic and normal rats are summarized. These examinations were carried out for the purpose of a general orientation, — and further detailed investigations should be made.

From table 68, showing the hemoglobin in the examined hypervitaminotic rats, — it is evident that there are considerable individual

Table 67.

Glycogen Content of the Livers of Hypervitaminotic and Normal Rats. (Experiment 37.)

Conditions of experiment	Rat no.	Glycogen content, mg/100 g
Basal diet (control)	1	82
	2	86
	3	82
	4	207
	Mean:	114
Basal diet + excess of vitamin A	1	10
	2	170
	3	50
	4	120
	5	10
	6	20
	7	20
	8	10
	9	20
	10	180
	11	75
	12	78
	13	60
	14	65
	15	185
	Mean:	72

variations in the hemoglobin values in rats given massive doses of vitamin A. In some cases distinct hypochromic anemia was observed in young and adult hypervitaminotic rats. In the majority of cases, however, the hemoglobin values fell within the range of the normal control rats, although on the whole they were lower than the average for normal rats. There appears to be no direct relation between the magnitude of the overdosage with vitamin A and the reduction in the hemoglobin values, as is evident from table 68. In some cases reduced hemoglobin values were associated with considerable hemorrhages which might possibly explain the anemia, but this was not always the case, as in some instances anemia was detected without any macroscopical hemorrhages being observed.

From table 68 it is further evident that the incidence of spontaneous macroscopical hemorrhages in hypervitaminotic rats was less in the present experiments than generally accepted. There appears to be no significant difference in the incidence of hemorrhages in young and adult animals.

A considerable reduction of the serum iron was only detected in one rat (35 γ%), in which case a marked anemia was also observed (see page 97).

In a previous investigation (Rodahl, 1949, 2) no abnormalities were detected in the differential blood counts in rats given excess of vitamin

Table 68.

Hemoglobin, and the Incidence of Macroscopical Hemorrhages in Hypervitaminotic Rats, Compared with Normal Control Rats.

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	Hemorrhage
Basal diet (control)	♀	87	28	105	5.2	1.0	0
	♀♂	93	33	112	5.5	1.0	0
	♂	64	24	102	-	-	0
	♂	45	23	95	-	-	0
	♂	45	23	100	-	-	0
	♂	47	23	97	-	-	0
	♀	67	30	102	-	-	0
	♀♂	77	30	104	-	-	0
	♀♂	68	33	104	-	-	0
	♂	233	50	104	-	-	0
	♂	169	50	102	-	-	0
	♀	21	73	105	-	-	0
	♀♂	26	73	112	-	-	0
	♀♂	38	73	104	-	-	0
	♀	29	73	110	-	-	0
	♀♂	26	73	97	-	-	0
	♂	Adult	-	106	5.2	1.0	0
	♀	Adult	-	100	5.2	1.0	0
	-	Adult	-	93	4.6	1.0	0
	-	103	45	93	-	-	0
	-	95	45	81	-	-	0
	-	88	45	102	-	-	0
	Mean:	-	-	101	5.1	1.0	-
Basal diet + vit. A-free liver oils	♂	76	46	91	5.7	0.8	0
	♂	97	46	108	5.6	1.0	0
	♀♂	93	46	107	5.2	1.0	0
	♂	59	29	107	-	-	0
	♂	59	29	102	-	-	0
	♂	104	11	108	-	-	0
	♂	96	11	102	-	-	0
	♀♂	70	30	104	-	-	0
	♂	82	30	107	-	-	0
	Mean:	-	-	104	5.5	0.9	-
Basal diet + excess of vit. A 10,000 I. U. vit. A daily	♀	67	58	100	-	-	0
	♂	83	58	104	-	-	0
	♀	77	58	102	-	-	0
	♀♂	69	53	91	-	-	0
	♂	96	19	93	-	-	0
	♂	87	19	112	-	-	0
	Mean:	-	-	100	-	-	-
15,000 I. U. vit. A daily	♀	49	26	89	5.1	0.8	0
	♀	44	26	97	5.0	1.0	0
	♀	53	26	88	5.1	0.9	0
	Mean:	-	-	91	5.1	0.9	-

Table 68 (cont.)

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	Hemorrhage
Basal diet + excess of vit. A (cont.)							
25,000 I.U. vit. A daily	♀	174	34	93	-	-	0
	♂	170	34	56	-	-	0
	♂	176	34	104	-	-	0
	♀	166	34	84	-	-	0
	♀	156	34	67	-	-	0
	Mean:	-	-	87	-	-	-
37,500 I.U. vit. A daily	♀	56	27	99	-	-	0
	♂	60	27	91	-	-	0
	♀	78	37	78	-	-	0
	♀	67	41	86	4.4	0.9	0
	♂	86	45	104	6.2	0.8	0
	♂	85	45	99	5.5	0.9	0
	Mean:	-	-	93	5.4	0.9	-
50,000 I.U. vit. A daily	♂	90	65	78	5.4	0.7	+ +
	♂	81	65	64	5.4	0.6	+
	♂	139	69	87	5.1	0.9	0
	♀	102	119	86	5.3	0.8	0
	♀	83	119	74	4.6	0.8	+
	♀	114	119	88	5.2	0.9	0
	♀	72	86	74	4.6	0.8	0
	♂	73	78	102	-	-	0
	♂	87	84	76	4.5	0.8	0
	♀	89	40	88	5.4	0.8	0
	♀	43	18	83	4.2	1.0	0
	♂	61	27	88	-	-	+
	♂	64	30	91	-	-	0
	Mean:	-	-	83	5.0	0.8	-
52,500 I.U. vit. A daily	♀	61	11	96	-	-	+ +
	♀	60	21	84	-	-	0
	Mean:	-	-	90	-	-	-
60,000 I.U. vit. A daily	♀	91	28	93	5.8	0.8	0
	♀	80	38	82	-	-	0
	♀	84	42	59	4.3	0.7	0
	♀	88	42	72	4.1	0.9	+
	Mean:	-	-	77	4.4	0.8	-
50,000—90,000 I.U. vit. A daily	♂	178	16	98	-	-	+ +
	♂	183	24	93	-	-	+
	♂	202	44	58	-	-	+ +
	♂	204	29	93	-	-	+ +
	♂	176	45	84	-	-	+ +
	♂	220	48	93	-	-	0
	♂	197	13	96	-	-	0
	♂	216	46	93	-	-	0
	♂	203	46	104	-	-	0
	♂	223	46	93	-	-	0
	♂	150	46	93	-	-	0
	Mean:	-	-	91	-	-	-

Table 68 (cont.)

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	Hemorrhage
Basal diet + excess of vit. A (cont.)							
100,000 I. U. vit. A daily	♂	163	4	103	-	-	0
	♂	157	4	107	-	-	0
	♀	146	8	98	-	-	0
	♀	160	8	100	-	-	0
	♀	153	12	96	-	-	0
	♀	171	12	87	-	-	0
	♀	163	16	96	-	-	0
	♀	180	16	86	-	-	+
	♀	170	20	93	-	-	0
	♀	161	20	95	-	-	0
	♀	174	20	95	-	-	0
	♀	149	28	70	-	-	+ +
	Mean:	-	-	94	-	-	-
Excess of vit. A given by subcutaneous injection							
10,000 I. U. vit. A daily	♀	100	33	89	-	-	+ +
	♀	100	33	102	-	-	0
	Mean:	-	-	96	-	-	-
Young from hypervitaminotic mothers							
Newborn	-	-	0	51	-	-	0
	-	-	0	73	-	-	0
21 days old	-	-	21	60	3.4	0.9	0
41 days old	-	-	41	82	-	-	0
	Mean:	-	-	67	-	-	-
Basal diet + excess of vit. A + vit. C							
25,000 I. U. vit. A daily	♀	80	40	106	-	-	0
	♂	96	40	104	-	-	0
	♀	84	40	104	-	-	0
	♂	68	40	106	-	-	0
	♂	81	40	112	-	-	0
	Mean:	-	-	106	-	-	-
50,000 I. U. vit. A daily	♀	40	29	102	5.1	1.0	0
	♀	41	35	108	-	-	0
	♂	51	38	99	-	-	0
	♀	46	75	102	-	-	0
	♀	45	20	87	4.8	0.9	0
	♂	48	20	82	4.8	0.9	0
	♀	50	20	90	-	-	0
	♀	46	20	80	-	-	0
	♀	49	20	86	-	-	0
	♀	59	21	86	-	-	+
	♀	53	21	90	-	-	0
	♂	41	21	73	-	-	0
	♂	40	21	87	-	-	0
	♂	59	21	76	-	-	0
	♂	83	21	90	-	-	+
	♂	70	21	93	-	-	(0)
	♂	98	33	102	-	-	0
	♂	99	33	82	-	-	0
	Mean:	-	-	90	4.9	0.9	-

Table 68 (cont.)

Conditions of experiment	Sex	Initial body weight, g	Duration of experiment, days	Hb %	Red blood cells, mill. per 1/1000 ml	Colour index	Hemorrhage
Basal diet + excess of vit. A + vit. C							
52,500 I. U. vit. A daily	♀	64	21	84	-	-	0
60,000 I. U. vit. A daily	♀	95	28	80	4.5	0.9	0
	♀	90	42	74	3.4	1.0	0
	Mean:	-	-	77	4.0	1.0	-
Rachitic diet + excess of vit. A							
50,000 I. U. vit. A daily	♀	49	7	88	-	-	+
	♀	51	11	85	-	-	+ +
	Mean:	-	-	87	-	-	-
Rachitic diet + excess of vit. A + vit. D							
50,000 I. U. vit. A daily	♀	49	43	85	-	-	0
	♂	54	19	68	4.3	0.8	0
	♂	67	33	88	5.5	0.8	0
	♂	69	40	100	-	-	0
	♂	72	68	79	4.4	0.9	0
	♀	57	88	70	4.6	0.8	0
	♂	73	88	79	-	-	0
	♂	92	90	65	4.6	0.7	0
	Mean:	-	-	79	4.7	0.8	-
80,000 I. U. vit. A daily	♀	84	10	79	-	-	0
	♀	68	10	88	-	-	0
	♂	81	9	79	-	-	+
	Mean:	-	-	82	-	-	-
Rachitic diet + moderate excess of vit. A + vit. D							
2,500 I. U. vit. A daily	♀	57	13	92	-	-	0
	♂	66	13	101	-	-	0
	♀	70	13	89	-	-	0
	♀	51	13	65	-	-	0
	Mean:	-	-	87	-	-	-
Rachitic diet	♂	66	18	90	4.1	1.0	0
Rachitic diet + vit. D							
	♂	76	30	100	-	-	0
	♀	70	13	99	-	-	0
	♀	96	13	99	-	-	0
	Mean:	-	-	99	-	-	-

T a b le 69.

Results of Differential Blood Counts, %, Average.

Conditions of experiment	Eosinophiles	Band forms	Polymorph.	Lymphocytes	Monocytes	No. of observations
Basal diet (control)	-	1 (1—0—1)	10 (10—12—9)	86 (90—79—89)	4 (1—9—2)	3
Basal diet + whale liver oil free of vit. A	1	-	10	90	-	1
Basal diet + excess of vit. A: 10,000 I. U. vit. A daily	1 (1—2—3—1—1)	1 (2—1—1—0—0)	15 (16—21—17 —15—6)	82 (83—78—77— 81—94)	2 (1—0—3—2—0)	5
15,000 I. U. vit. A daily	-	1	5	95	1	1
50,000 I. U. vit. A daily	1 (0—2—1)	4 (5—1—6)	18 (14—27—12)	75 (80—71—74)	3 (1—0—7)	3
50,000—90,000 I. U. vit. A daily	1 (1—1)	1 (1—0)	19 (17—22)	78 (78—78)	2 (4—0)	2
Basal diet + excess of vit. A + vit. C	1 (0—2—1—1—0 —1—0—3—1)	1 (2—2—0—1—2 —1—2—1—2)	20 (16—18—26— 18—19—24— 18—23—18)	77 (80—76—73— 78—80—75— 80—74—79)	1 (3—3—1—1—0 —0—0—0—1)	9
Rachitic diet	1	1	21	70	7	1
Rachitic diet + vit. D	1 (0—1)	1 (1—0)	17 (15—18)	79 (79—78)	5 (5—4)	2
Rachitic diet + vit. D + excess of vit. A	- (0—1—0)	3 (1—5—2)	36 (20—33—56)	55 (78—59—41)	2 (2—3—3)	3

A (15,000—30,000 I. U. daily). In the present investigation (see table 69), no significant difference was detected in the differential blood count in hypervitaminotic rats compared with the normal control rats, except for a slightly reduced number of lymphocytes and increased number of polymorphonuclear leucocytes in rats given massive doses of vitamin A. Similar conditions were found in the group given vitamin C in addition to the excess of vitamin A. In a group given excess of vitamin A in addition to rachitic diet and vitamin D, there was a marked reduction in the number of lymphocytes and a corresponding increase in the number of polymorphonuclear leucocytes.

The total base in the serum was determined in 8 hypervitaminotic rats, all of which showed normal values (149—151 milliequivalents/1).

The potassium content of the serum was 4.5—5.7 milliequivalents/1 in hypervitaminotic rats, and 4.9 in normal rats (see page 67).

There was no significant difference in the urea content of the blood in hypervitaminotic rats and in normal rats (49—59 mg/100 ml, as against 43—53 in normal rats).

The fasting blood sugar was in all cases, except three, between 83 and 109 in the hypervitaminotic rats, as against 88 in normal control rats. In one rat, which was moribund, it was only 60 mg/100 ml, and in two cases it was 165 mg/100 ml.

The calcium content of the serum was determined in one case and found to be 10.1 mg/100 ml which is in agreement with previous findings (Rodahl, 1949, 2). The phosphorus content was determined in three cases and was found to be 5.1, 5.4 and 5.9 mg/100 ml.

B. Hypervitaminosis A in Mice.

1. *The Effect of Local Application of Vitamin A Concentrate.*

Experiment 38.

The effect of local application of vitamin A concentrate in the form of bear liver oil on the skin, was first studied in 5 normal adult white mice.

One drop of saponified bear liver oil, corresponding to approximately 20,000 I. U. vitamin A was daily smeared on the skin between the shoulder blades on a spot where the hair had been cut off with a pair of scissors. At the end of 14 days marked alopecia was observed on and around the area where the oil had been applied. The treatment was then discontinued, and the mice were observed for a period of 4 weeks.

At the end of one month from the beginning of the experiment, all the mice showed typical signs of hypervitaminosis A, — identical to those observed in rats receiving excess of vitamin A orally. The symptoms were exopthalmus, alopecia, soreness and swelling of the palpebrae, and loss of hair around the eyes, soreness around the mouth and nose as well as distinct clinical signs of fractures of the hind legs. Six days later one of the mice died.

One month after the separation of the bear liver oil the symptoms were still marked, and one of the mice had a large tumour on the back underneath the place where the oil had been applied. This mouse was killed and examined. There was marked alopecia around the right eye, which was practically closed by the swelling of the palpebrae. There were blood crusts and small hemorrhages around the right eye and the right ear. The hind legs appeared paralyzed. The tumour on the back was 4 mm in diameter, and was attached to the skin and the subcutaneous tissue. By microscopical examination it was found to be an encapsuled abscess containing a large number of neutrophil granulocytes and necrotic tissue. There was marked alopecia and diffuse hemorrhages and inflammation with pronounced capillary reaction in the surrounding tissue. Similar abscesses were observed in three out of the 5 mice.

By postmortem examination similar findings were made in the organs as described for the hypervitaminotic rats.

As the 5 mice were kept together in the same cage, it was thought possible that they might have licked the oil from the backs of each other with the result of hypervitaminosis A through ingestion of small quantities of saponified bear liver oil rich in vitamin A.

The experiment was therefore repeated with 5 other mice separated in individual cages. Otherwise the conditions of the experiment were identical with the previous experiment with the exception that in this case unsaponified bear liver oil was used in the same way as previously described. Great care was taken that the oil should not be spread outside the smeared area which was approximately 1 cm in diameter.

At the end of 10 days one of the mice died, and by postmortem examination signs of peritonitis were found. At that time all 5 mice showed the same symptoms as mentioned before with marked loss of hair where the oil had been smeared and also over large areas of the back and the neck, as well as distinct eye symptoms. At the end of 18 days from the beginning of the experiment 4 out of the 5 mice had died.

Histological examination of the liver, kidneys, and adrenals from one of the mice revealed similar findings as described for the hypervitaminotic rats.

From this experiment it may be concluded that local application of bear liver oil on the skin, causes symptoms of hypervitaminosis A in mice, even when no oil is ingested orally.

2. *The Effect of Oral Administration of Vitamin A Concentrate.*

Experiment 39.

In a final experiment excess of vitamin A in the form of whale liver oil concentrate was given orally to mice in order to determine the approximate gross dose of vitamin A sufficient to cause toxic symptoms in mice.

For this purpose 8 male albino mice with initial body weights from 16—20 grams were used, and divided into 4 groups which were given varying doses of the same whale liver oil concentrate (200,000 I. U. vit. A/g), which had been used in the previously described experiments on rats.

One group was given an average of 1,250 I. U. vit. A daily (60 I. U. vit. A/g body weight), a second group 2,500 I. U. vit. A daily (130 I. U./g body weight), a third group 5,000 I. U. vit. A daily (300 I. U./g body weight), and a final group 10,000 I. U. vit. A daily (600 I. U./g body weight).

The doses proved lethal in the animals in all cases at the end of periods from 9 to 34 days. Thus 60 I. U./g body weight daily proved lethal after 16 and 27 days; 130 I. U./g body weight daily after 16 and 34 days; 300 I. U./g body weight daily after 16 days; and 600 I. U./g body weight at the end of 9 days. At the same time a reduction in the weight was observed. The relation between the gross vitamin A dose, the time of survival, and the loss of weight in the mice given whale liver oil concentrate is given in table 70.

From table 70 it is evident that the loss of weight increased by increasing doses of vitamin A. The average weight graphs for these mice compared with normal controls are given on page 139.

In the two mice given 60 I. U. vit. A/g body weight, one appeared only slightly affected two days prior to death. In the second mouse soreness was observed at the base of the tail on the 4th day, and loss of hair around the mouth on the 13th day. From the 17th day it looked particularly unwell, with a scruffy pelt, and it suffered from soreness in the skin and marked alopecia.

In the group given 130 I. U./g body weight daily, eye symptoms with exophtalmus and swelling of the palpebrae were observed in one of the mice on the 3rd day, and soreness around the eyes on the 7th day. Two days later one of the eyes was

Table 70.

Relation Between the Gross Vitamin A Dose, the Time of Survival, and Loss of Weight in Mice Given Whale Liver Oil Concentrate (Experiment 39).

Gross dose of vit. A		Mouce no.	No. of days until death occurred	Loss of weight		
I. U./day	I. U./g body weight/day			Total, g	g/day	
					Individual	Average
1,250	60	1	16	0.0	0 00	0.05
		2	27	3.0	0.10	
2,500	130	3	16	1.5	0.10	0.06
		4	34	0.8	0.02	
5,000	300	5	16	3.0	0.18	0.14
		6	16	1.5	0.10	
10,000	600	7	9	3.0	0.43	0.46
		8	9	3.5	0.50	

completely closed. Marked alopecia was observed from the 11th day. These symptoms gradually became more pronounced until the mouse appeared moribund and died on the 34th day. In the other mouse, soreness around the mouth and alopecia were observed on the 9th day. Two days later there was marked swelling of the eyes, one of which was practically closed. The symptoms rapidly became worse, and the mouse died on the 16th day.

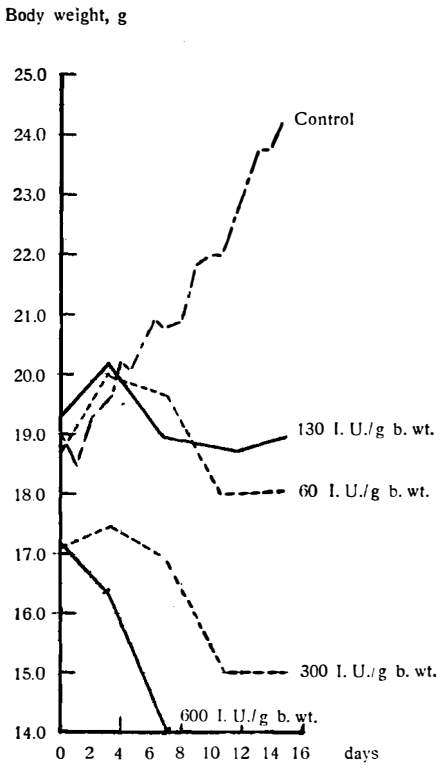
In the group receiving 300 I. U. vit. A/g body weight daily the above mentioned symptoms occurred earlier and rapidly became more pronounced than in the previous group. In this group diarrhea was also observed, and in one of the mice fractures were diagnosed.

In the final group receiving 600 I. U. vit. A/g body weight the toxic symptoms occurred even more pronounced and in a more rapid succession, although no fractures were observed in this group.

By postmortem examination similar findings as in the hypervitaminotic rats were made. There was marked hyperemia and scattered subcutaneous and visceral hemorrhages. In the two mice receiving the largest dose of vitamin A, considerable subcutaneous hemorrhages as well as bleeding from the genital organs were found and the adrenals appeared enlarged. In no case was there detected signs of infection, enteritis or pneumonia.

Microscopical examination of the liver, kidneys, adrenals and pancreas from two of the mice, revealed similar findings as previously described for the hypervitaminotic rats, such as marked hyperemia, slight degeneration of the renal tubules, scattered red blood cells outside the capillaries in the kidney, and erythrocytes in the space of Bowman's capsule. In the liver stained by sudan III, a large number of sudanophil droplets were observed in and, in particular, between the liver cells.

By X-ray examination of the long bones, similar findings were made as described for the hypervitaminotic rats.



Graph No. 15. Average weight graphs for mice given excess of vitamin A, compared with normal control mice.

Summary of Results. From these experiments it may be concluded that excess of vitamin A is toxic to mice when given orally, and that doses from 60 to 600 I. U. vit. A per gram body weight proved lethal in all cases. The loss of weight was found to increase by increasing doses of vitamin A, and the symptoms produced in mice by oral administration of vitamin A concentrate were identical with those observed in mice when the concentrates were applied locally on the skin, which again were identical with the symptoms observed in rats given excess of vitamin A orally.

C. Hypervitaminosis A in Guinea Pigs.

As is evident from the previously described experiments, the symptoms produced in rats given excess of vitamin A resembled those observed in scurvy in experimental animals. As rats are not normally liable to develop scurvy because of their endogenous synthesis of ascorbic acid, a clear study of the relationship between hypervitaminosis A and experimental scurvy could not be carried out in rats.

It was therefore considered desirable to examine the effect of excess of vitamin A in guinea pigs, which depend on exogenous supply of vita-

min C, and to investigate the relation between hypervitaminosis A and experimental scurvy in these animals. In this connection it was also of interest to study the effect of excess of vitamin A in guinea pigs kept on a scorbutic diet, — and finally to ascertain whether additional doses of vitamin C offered any protection against the injurious effect of excess of vitamin A.

Experimental Animals, and Dosage.

In the following experiments, guinea pigs of various age and of both sexes were used, with initial body weights varying from 55 to 330 grams. They were all taken from the main stock of animals which had been kept on the usual adequate basal diet consisting of oats, hay, cabbage, turnips, carrots, milk and water. Prior to the commencement of each experiment the experimental animals were kept separated from the main stock of animals for several days, during which time they were made accustomed to the feeding technique, and the weight gain was observed.

As a source of vitamin A, the usual whale liver oil concentrate (200,000 I. U. vit. A/g) was used in some of the experiments. In order to avoid dosage of unduly large quantities of oil, a highly concentrated source of vitamin A was used in some of the experiments, consisting of a purified shark-liver oil dissolved in a minimum of peanut-oil, with a potency of 640,000 I. U. vit. A/g. This oil contained practically no vitamin D. In some of the experiments the control animals were given peanut oil in equivalent amounts to the vitamin A oil given to the experimental animals. The vitamin oil was either given by dropping pipet, or by a specially constructed catheter.

1. *Preliminary Investigations.*

Experiment 40.

For the purpose of a preliminary investigation 6 young guinea pigs of approximately the same age were used, with initial body weights between 90 and 120 grams. All these animals were kept under identical conditions.

One of the guinea pigs was used as control and was given the ordinary basal diet only. A second animal was given excess of vitamin A in addition to the basal diet in the form of the usual whale liver oil concentrate in amounts corresponding to 50,000 I. U. vitamin A daily (approximately 550 I. U. vit. A/g body weight). A third animal was given 50 mg ascorbic acid by subcutaneous injection (ampules pro injectione) in addition to the usual basal diet and the same dose of vitamin A as given to guinea pig no. 2. A fourth animal was given a scorbutic diet without any supply of ascorbic acid whatever. A fifth animal was given excess of vitamin A in addition to the scorbutic diet, in the form of the previously mentioned whale liver oil concentrate in amounts corresponding to 50,000 I. U. vit. A daily (approximately 415 I. U./g body weight). The whale liver oil was given by dropping pipet. Finally a sixth animal was given excess of vitamin A by subcutaneous injection.

Shortly after the beginning of the experiment loss of weight was observed in all cases, except for the control. On the second day of the experiment the animal given scorbutic diet accidentally fractured one of its hind legs. On the 6th day a limping gait was observed in the three guinea pigs receiving excess of vitamin A

orally. The following day all experimental animals suddenly died, except the guinea pig given ascorbic acid in addition to the excess of vitamin A, which appeared to be in good health, and the animal given excess of vitamin A by subcutaneous injection. By this time the control animal had gained 12 grams in weight since the commencement of the experiment, while all the other guinea pigs except the one given excess of vitamin A by subcutaneous injection had lost weight, as shown in table 71.

Table 71.

Loss of Weight in Guinea Pigs During the First Week of the Experiment (Experiment 40).

Conditions of experiment	Loss of weight, g
550 I. U. vit. A/g body weight daily	8.5
550 I. U. vit. A/g body weight + 50 mg ascorbic acid daily	0.2
Scorbutic diet	6.5
Scorbutic diet + 415 I. U. vit. A/g body weight daily	24.5

On the 8th day of the experiment the animal given vitamin C in addition to excess of vitamin A also died, although it had appeared quite normal the previous day. By postmortem examination the lungs showed signs of pneumonia. Otherwise no significant pathological findings were made. By microscopical examination of the liver, kidneys, adrenals and pancreas, marked hyperemia was observed.

By postmortem examination of the guinea pig given excess of vitamin A (no. 2), the pelt was scruffy, and there was general alopecia. There was hyperemia in the internal organs, and in the lungs signs indicating pneumonia were found. The liver, kidney and adrenals were speckled on the surface, and masses of coagulated blood was found around one of the adrenals. The bones appeared abnormally brittle and were easily broken when grasped with an ordinary forceps. By microscopical examination of the liver, kidneys and adrenals marked hyperemia was found. In the liver and kidneys, scattered red blood cells were seen outside the capillaries in some places, and there was slight degeneration of some of the renal tubules (1st convoluted tubules and collecting tubules), the cells showing marked swelling and granularity.

In the guinea pig given scorbutic diet, the pelt appeared scruffy, with a slight degree of alopecia. There was hyperemia in the internal organs, and a large hemorrhage on the surface of the left adrenal, involving ⅔ of the gland. In the lungs there were signs resembling pneumonia. Microscopical examination of the liver, kidneys and adrenals revealed marked hyperemia.

In the guinea pig given excess of vitamin A in addition to the scorbutic diet, marked hyperemia and scattered hemorrhages as well as signs indicating pneumonia were found by postmortem examination. The bones appeared abnormally brittle as described for guinea pig no. 2. By microscopical examination of the liver, kidneys and adrenals marked hyperemia was observed. In the liver and kidneys scattered red blood cells were seen outside the capillaries. In the kidneys there was degeneration of some of the convoluted and collecting tubules, which contained amorphous

masses of a calcium-like substance stained dark blue with hematoxylin-eosin. In one of the adrenals, small hemorrhages were observed in the cortex and numerous mitoses were seen in the cortical cells. By sudan staining dense deposits of large sudanophil droplets were observed in the adrenal cortex. No sudanophil droplets were seen in the kidney, and only few in the liver.

The final guinea pig, no. 6, which had an initial body weight of 117 grams, was injected with 20,000 I. U. vit. A and observed during a period of 6 days, at the end of which time the same dose was again injected and a further observation over a 6 day period took place, when it was once again injected with the same amount of vitamin A. Following the injection a sudden development of cramps in the fore legs was observed, and it eventually died (probably as a result of fat embolus). A blood sample was collected in which the hemoglobin was 79 %, and the ascorbic acid content 0.00 mg/100 ml.

Following the first injection of vitamin A a short-lasting weight fall was observed, — followed by normal weight gain.

By postmortem examination, marked alopecia was observed, as well as fracture of two ribs. Otherwise no pathological findings were made.

By microscopical examination of the liver, kidneys, adrenals, pancreas, testes and bones the following positive findings were revealed: In the kidneys a few red blood cells were seen in the space of Bowman's capsule, and in the adrenals a large number of vacuoles were seen in the cortex. By sudan staining of the liver, no sudanophil droplets were detected, while in the adrenals the entire cortex was densely filled with deposits of sudanophil droplets. Otherwise no significant pathological findings were made.

Although no definite conclusion could be reached from this preliminary experiment, it appeared that a combination of the scorbutic diet and excess of vitamin A proved more injurious to the guinea pigs than excess of vitamin A or scorbutic diet alone. From these observations it further appears that excess of vitamin A is also toxic to guinea pigs when injected subcutaneously.

2. *The Effect of Excess of Vitamin A.*

From the preliminary experiments, no definite conclusion could be reached as to the clinical picture of hypervitaminosis A in guinea pigs, as all the animals given excess of vitamin A orally died with signs of pneumonia shortly after the commencement of the experiment. The purpose of the following experiments was therefore to examine more closely the effect of various massive doses of vitamin A in guinea pigs of different ages.

Experiment 41.

For the purpose of studying the effect of excess of vitamin A in very young guinea pigs, three guinea pigs of the same litter, with initial body weights of 55—73 grams were used. They were all given the usual adequate basal diet in unlimited quantities, and lived under identical conditions.

One of the animals was used for control purposes (no. 7), the remaining two animals were given two drops of the usual whale liver oil concentrate, corresponding to 10,000 I. U. daily.

One of these animals (no. 8), which had an initial body weight of 55 grams, and consumed 180 I. U. vit. A/g body weight daily, died at the end of 6 days. It appeared weak and scruffy already on the 4th day, and two days later there was soreness of the eyes. The average loss of weight was 1.5 grams daily.

The second experimental animal (no. 9) which had an initial body weight of 73 grams, and consumed approximately 130 I. U. vit. A/gram body weight daily, died at the end of 12 days having appeared weak and scruffy from the 6th day. The average daily loss of weight was 0.8 grams.

The control animal had an initial body weight of 68 grams, and gained an average of 5 grams daily (see weight graph on page 144).

X-ray examination of the long bones in guinea pig no. 9 revealed abnormally thin bone shafts and cortex, fracture of the proximal end of the tibia and dense lines of calcification at the border of the epiphyses. By postmortem examination alopecia was seen around the anus and the mouth. There were scattered subcutaneous hemorrhages, as well as free blood in both knee joints, where coagulated blood was found when the joints were opened. The inguinal lymph glands were swollen, the pancreas and the lungs were fiery red, but there were no signs of pneumonia. The bones were very brittle (see page 141). In the urine the Heller's reaction was negative.

Microscopical examination of the liver, kidneys, adrenals and pancreas revealed hyperemia and scattered red blood cells outside the capillaries in the liver and kidneys. In the liver a large number of vacuoles were seen, and swelling of the Kupffer cells. In the kidneys a few red blood cells were seen in the space of Bowman's capsule, as well as degeneration of some of the tubules, which were filled with necrotic cells and deposits of a calcium-like substance. In the adrenals, there was hyperemia, and the cortical cells in the zona fasciculata appeared irregularly arranged, and contained a large number of vacuoles.

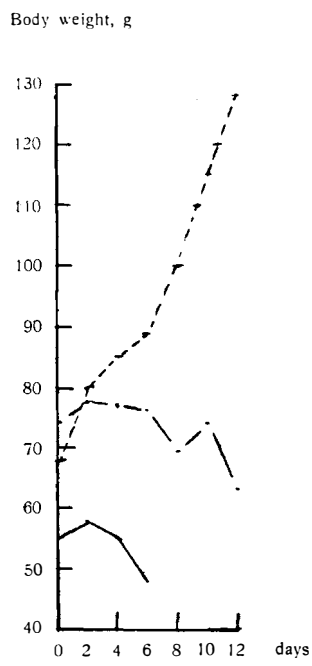
The ash content of the femur calculated on a dry basis in guinea pig no. 9 was 45 % and the calcium and phosphorus contents of the ash were 34.1 % and 20.0 % respectively.

From this experiment it appears that very young guinea pigs are very sensitive to overdosage with vitamin A. Thus 130 I. U. vit. A/g body weight daily proved fatal in 12 days, and 180 I. U./g in 6 days.

Experiment 42.

The purpose of this experiment was to study the effect of relatively moderate excess of vitamin A on the weight gain in guinea pigs, in an attempt to determine the approximate minimum dose of vitamin A sufficient to interfere with the normal weight gain in these animals. For this purpose three normal guinea pigs with initial body weights of 100—110 grams were used.

One of the animals was used as a control (no. 10), and received ordinary adequate basal diet in unlimited quantities, with an addition of two drops of peanut oil daily by dropping pipet, during a period of 30 days, at the end of which it was killed and examined. It remained free of symptoms throughout the experiment, and gave normal postmortem findings. The average daily weight gain was 5.0 grams. The hemoglobin was 100 %, and the ascorbic acid content of the serum was 0.20 mg/100 ml.



Control (Guinea pig no. 7)

130 I. U. vit. A/g body weight daily. (Guinea pig. no. 9)

180 I. U. vit. A/g body weight daily. (Guinea pig. no. 8)

Graph No. 16. Individual weight graphs for young guinea pigs given excess of vitamin A, compared with the normal control.

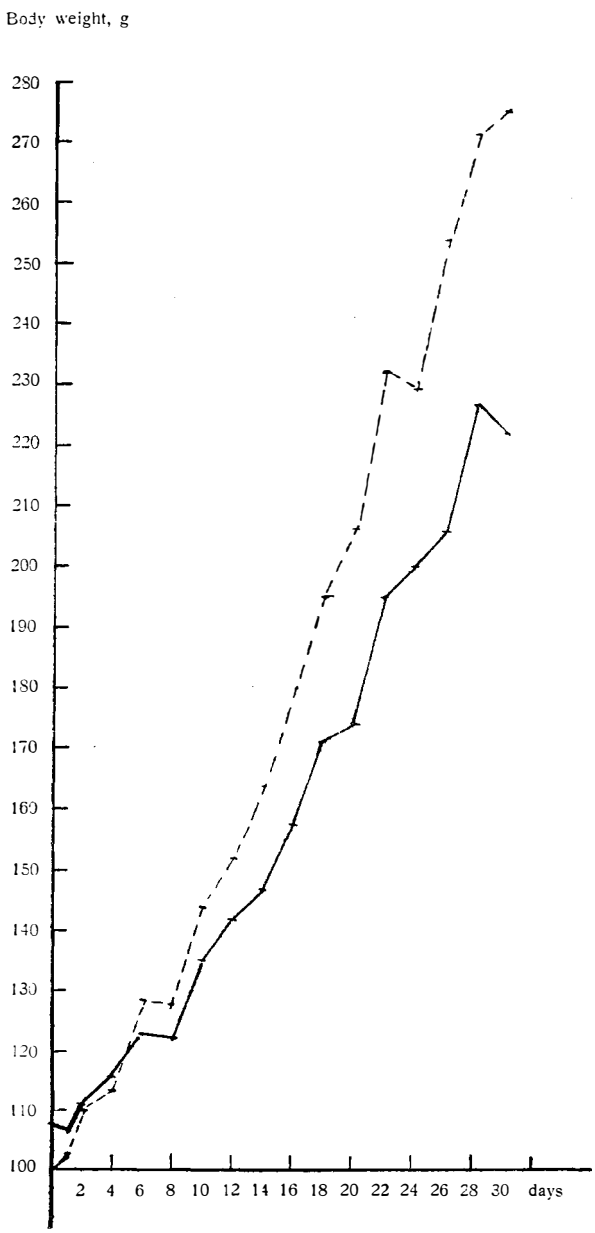
Microscopical examination of the liver, kidneys, adrenals, thymus and testes showed normal findings, as did microscopical examination of the teeth and bones. By sudan staining of the liver, no deposits of sudanophil droplets were found, while sudan staining of the adrenals showed deposits of sudanophil droplets throughout the cortex.

The ash content of the femur calculated on a dry basis was 53.2 %, and the calcium and phosphorus contents of the ash were 31.1 % and 17.0 % respectively.

The second guinea pig (no. 11) received in addition to the basal diet, two drops of whale liver oil concentrate, furnishing 10,000 I. U. vit. A daily, or 87 to 60 I. U. vit. A/g body weight, during a period of 15 days. After this the dose was increased to 20,000 I. U. daily, corresponding to 120 to 88 I. U. vit. A/g body weight, for a period of 15 days, at the end of which it was killed and examined. The average weight gain was 3.8 grams daily (see graph no. 17). It appeared quite healthy, and by postmortem examination, no pathological findings were made. There were no fractures, no alopecia, or soreness, no changes in the pelts, no hemorrhages, and no pathological findings in the internal organs.

The blood coagulated normally. There was no increase of the serum colour. The hemoglobin was 100 %, and the ascorbic acid content of the serum was 0.14 mg/100 ml.

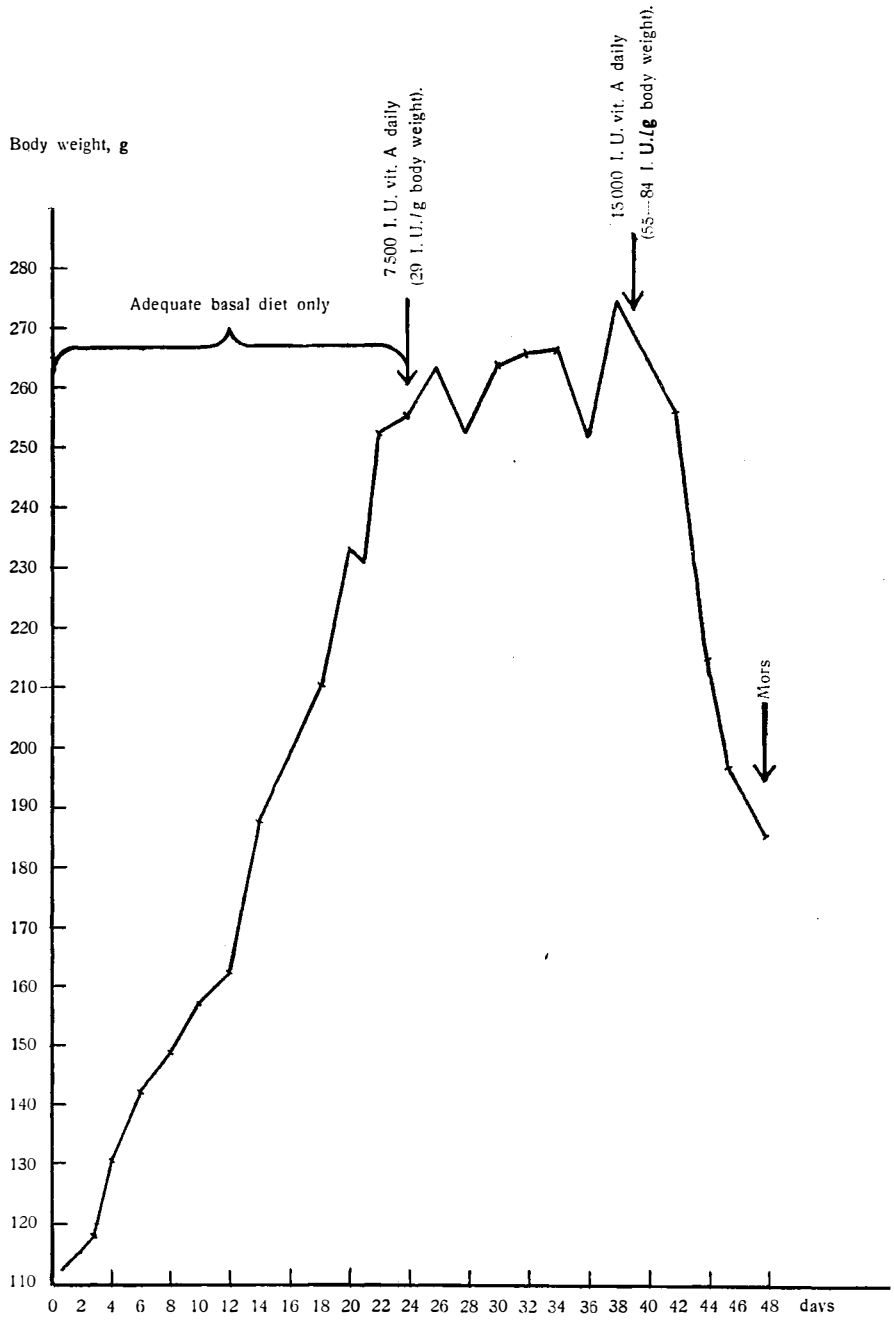
By microscopical examination of the liver, kidney, adrenal, pancreas, thymus and testis, no significant pathological findings were made. By sudan staining of the liver, a few sudanophil droplets were found, while sudan staining of the adrenals showed dense deposits of sudanophil droplets throughout the cortex, except for a narrow band at the periphery. In the bones, similar findings as previously described for the hypervitaminotic rats were made. The compact bone was remarkably thin, with large Howship's lacunae.



Graph No. 17. Weight graph of guinea pig. no. 11 receiving 10,000—20,000 I. U. vit. A. daily (60—120 I. U./g body weight), compared with the control (guinea pig no. 10).

The ash content of the femur calculated on a dry basis was 53.4 %, and the calcium and phosphorus contents of the ash were 24.9 % and 17.7 % respectively.

The third guinea pig (no. 12) had an initial body weight of approximately 110 grams. Prior to the experiment it had been given nothing but the ordinary adequate basal diet during a period of 23 days, during which time the average daily weight gain had been 6.3 grams. At the commencement of the experiment it weighed 254 grams. It was then given one drop daily of a highly purified and concentrated shark liver oil (see page 140) dissolved in peanut oil, furnishing



Graph No. 18. Weight graph for guinea pig no. 12 in relation to doses of vitamin A.

7,500 I. U. vit. A daily, in addition to the usual basal diet. This dose (29 I. U. vit. A/g body weight), was given during a period of 15 days, during which time the average daily weight gain was 1.9 grams. The vitamin A dose was then increased to 15,000 I. U. vit. A daily (55—84 I. U. vit. A/g body weight). Following this increase of the vitamin A consumption, a rapid weight fall occurred, which continued steadily until the animal died 10 days after the dose had been increased. During this latter period, the average daily loss of weight was 9.5 grams (see weight graph no. 18).

By postmortem examination, alopecia and soreness around the mouth was observed. There was hyperemia, and subcutaneous hemorrhages over the abdomen, as well as bleeding in one of the knee joints and in the musculature of the hind legs. There were no signs of pneumonia. Microscopical examination of the internal organs revealed no significant pathological findings. By sudan staining of the liver, a few sudanophil droplets were found, and in the adrenals only slight deposits of sudanophil droplets were seen in the cortex. In the bones, similar findings as previously described for the hypervitaminotic rats were detected.

The ash content of the femurs was 52.6 % calculated on a dry basis, the calcium content of the ash was 34.6 %, and the phosphorus content: 19.4 %.

From this experiment it may be concluded that excess of vitamin A is toxic to young guinea pigs when given in amounts between 50 and 100 I. U. per gram body weight.

Experiment 43.

In a further experiment another young guinea pig (no. 13), with an initial body weight of 134 grams, and whose weight increase had been observed during a period of 12 days prior to the commencement of the experiment, was than given excess of vitamin A in the form of whale liver oil concentrate in amounts corresponding to 50,000 I. U. vit. A daily, by dropping pipet (370 I. U. vit. A/g body weight).

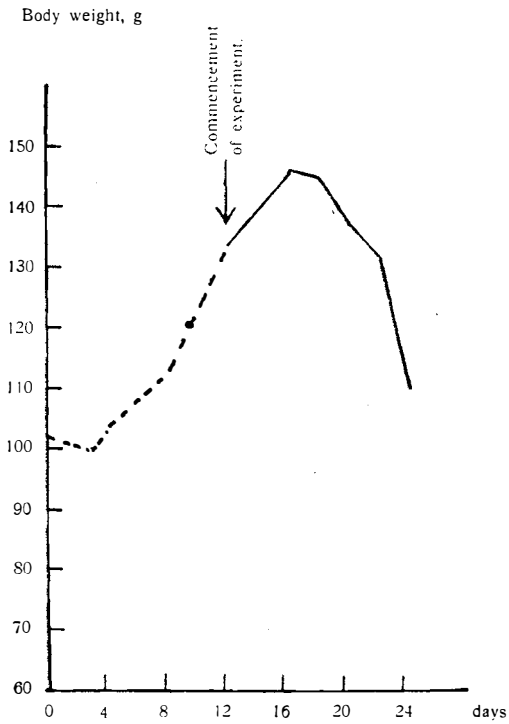
From the third day, the animal appeared unwell, showing lack of activity, and the pelt looked scruffy. From the 4th day the weight fell steadily (see graph no. 19). On the 6th day fracture of the right fibula was clinically diagnosed and later verified by X-ray examination.

Two days later the right knee joint appeared painful and swollen. The animal cried by touching of the limb. X-ray examination revealed, apart from the previously described fracture of the fibula, disunion of the epiphysis of the tibia, as well as a densely calcified line at the epiphyseal border of the tibial metaphysis.

The following day, both knee joints appeared swollen and painful. On the 10th day the fore legs were also affected, the joints being painful and swollen. Soreness around the nose was also observed. The following day these symptoms became more prominent. At the same time eye symptoms similar to those observed in hypervitaminotic rats, occurred. On the 12th day the animal was found moribund, lying motionless on its side, being unable to stand on its feet. There were clonic cramps in the fore legs, occurring at intervals of a few minutes.

It was then killed and examined after blood samples had been taken. The hemoglobin was 89 %, red blood cells 5.0 millions per 1/1000 ml, color index 0.9. The blood coagulated within 5 minutes.

By postmortem examination, subcutaneous hemorrhages were observed in the left axilla, as well as in the inguinal regions. Free blood was observed in both knee joints. The liver appeared fatty on the cut surface. Several greyish-yellow coloured spots were seen on the surface of the left kidney.



Graph No. 19. Weight graph of guinea pig no. 13 given excess of vitamin A (370 I. U./g body weight daily). Dotted line indicates weight prior to the commencement of the experiment. (Experiment 43).

By microscopical examination of the liver, kidneys, adrenals and the teeth, marked hyperemia was observed. A few red blood cells were seen outside the capillaries in the liver and kidney, and erythrocytes were also seen in the space of Bowman's capsule in a few places. There was degeneration of some of the renal tubules, some of which contained deposits of calcium.

Marked changes appeared in the teeth. There was pronounced hyperemia in the pulp, where vacuoles and scattered hemorrhages were seen, with complete irregularity of the odontoblasts, which in some places were represented by heaps of cells chaotically arranged.

X-ray examination of the long bones at the conclusion of the experiment revealed similar roentgenological bone changes as described for the hypervitaminotic rats, i. e. abnormal thinning of the bone shafts and the cortical shadow. A densely calcified line was observed at the epiphyseal borders of the tibial metaphyses. There were fractures of the distal ends of the left radius and ulna, as well as the proximal metaphyses of the right tibia and fibula, and disunion of the epiphysis.

The ash content of the femur was 39.6 %, calculated on a dry basis, the calcium content of the ash was 33.8 % and the phosphorus content 18.9 %.

Experiment 44.

In this experiment two young guinea pigs (nos. 14 & 15) were given 17,500 I. U. vit. A daily in the form of vitamin A alcohol dissolved in a minimum of peanut oil, whilst a third animal (no. 16) of approximately the same age was used as a control.

One of the guinea pigs (no. 14) which had an initial body weight of 100 grams and which received 175—220 I. U. vit. A/g body weight, died at the end of 9 days, having lost an average of 2.0 grams daily from the beginning of the experiment (see graph no. 20). At the end of 6 days, soreness around the eyes was observed, and two days later the general condition of this animal was very poor.

By postmortem examination, the following pathological findings were made: Soreness and alopecia around the mouth, scattered subcutaneous hemorrhages in the inguinal regions, axillae and the neck, as well as hemorrhages in the muscles of the right hind leg and around the right knee. There was marked hyperemia. The lungs were fiery red, and the bones were brittle.

By microscopical examination of the internal organs and bones, the following findings were made:

Liver: By sudan III staining a large number of sudanophil droplets were seen in and between the liver cells, particularly in the Kupffer cells.

Kidneys: Marked hyperemia. Red blood cells were seen outside the capillaries and in the space of Bowman's capsule. There was degeneration of the convoluted as well as the collecting tubules, some of which were filled with a calcium-like substance, stained dark blue by hematoxylin-eosin.

Adrenals: An accessory adrenal was seen. There were a large number of vacuoles in the adrenal cortex, where, by sudan staining, massive deposits of sudanophil droplets were seen.

Pancreas: Hyperemia.

Lung: Hyperemia.

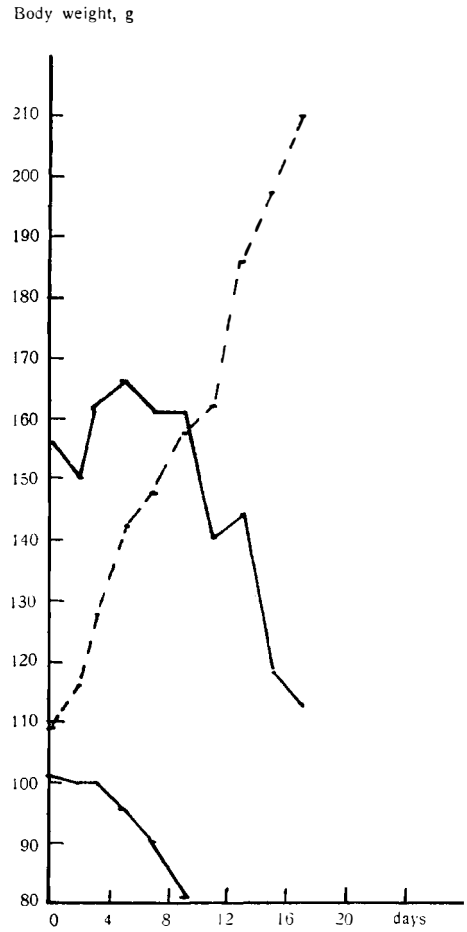
Bones (tibia): Marked hyperemia, irregularity of the cartillage and irregularly arranged bone. There was periostal hemorrhage. The compact bone had completely disappeared in some places, there was richness of calcium and enlarged Howship's lacunae, destruction of bone lamels, substituted by connective tissue in which scattered red blood cells were seen.

In the costochondral junction, marked hyperemia was seen. The compact bone had completely disappeared in several places and was substituted by connective tissue. The bone was chaotically arranged, with destruction of the spicules, large Howship's lacunae, periostal hemorrhages and signs of increased osteoclastic activity.

The ash content of the femur calculated on a dry basis was 48.8 % and the calcium and phosphorus contents of the ash were 30.7 % and 18.5 % respectively.

The second guinea pig in this group (no. 15), had an initial body weight of 155 grams and received 110—145 I. U. vit. A/g body weight daily. It died at the end of 17 days, after having lost considerable weight during the last 8 days of the experiment. The average daily loss of weight throughout the experiment was 3.0 grams. On the 13th day it looked weak and scruffy, and the following day the general condition was considerably worse, there were pains in the hind legs, and the eyes were sore, swollen and practically closed. Two days later it appeared moribund, being practically unable to walk, both hind legs appearing paralysed. At the same time alopecia and soreness around the mouth was also observed. X-ray examination of the long bones revealed detachment of the proximal tibial epiphyses, and densely calcified lines at the borders of the epiphyses.

Alopecia around the mouth was found by postmortem examination, together with scattered subcutaneous hemorrhages, hemorrhages in the muscles of the hind legs, and free blood in the knee joint. There was also swelling of the costochondral junction as well as fracture of one of the ribs (see ill. 47).



Control. (Guinea pig no. 16).

Guinea pig no. 15. (110—145 I. U. vit. A/g body weight daily)

Guinea pig No. 14. (175—220 I. U. vit. A/g body weight daily)

Graph No. 20. Weight graphs of guinea pigs given excess of vitamin A alcohol, compared with the normal control. (Experiment 44).

By microscopical examination of the internal organs and the bones, similar findings were revealed as described for the previous guinea pig, no. 14, although sudan staining of the liver showed practically no deposits of sudanophil droplets, and in the adrenal cortex considerably less, than in the previous animal.

The ash content of the femur calculated on a dry basis was 51.8 %, and the calcium and phosphorus contents of the ash were 30.3 % and 18.2 % respectively.

The control guinea pig (no. 16), which had an initial body weight of 108 grams, gained an average of 6 grams daily, and remained normal throughout the experiment.

From this experiment it appears evident that the injurious effect of excess of vitamin A alcohol is identical with that of equivalent amounts of vitamin A given in the form of purified whale liver oil concentrate, used in previous experiments.

3. *Hypervitaminosis A and Scurvy.*

From the previously described experiments on young guinea pigs given excess of vitamin A, it appears evident that the findings in some cases resemble scurvy, although no definite conclusion could be reached from these experiments as to the relation between hypervitaminosis A and scurvy in guinea pigs.

The purpose of the following experiments was therefore to examine more closely the lesions of hypervitaminosis A as compared with those of experimental scurvy, and to ascertain to what extent the condition of hypervitaminosis A might aggravate the condition of scurvy in guinea pigs.

Experiment 45.

In this experiment 7 guinea pigs of different ages were used, with initial body weights varying from 130 to 350 grams. One of the animals was used as a control, the remaining 6 were divided into three groups, each consisting of two animals. It was so arranged that in each group one of the younger, and one of the older animals were used in order to obtain some idea of the importanc of the age of the animals with regard to their resistance against overdosage with vitamin A.

One of the guinea pigs with an initial body weight of 327 grams was used as a control and was given the usual adequate basal diet in unlimited quantities.

One group of two guinea pigs (initial body weights: 296.5 and 196.5 g) were given 50,000 I. U. vitamin A daily, in addition to the basal diet, in the form of whale liver oil concentrate orally, with the aid of a specially constructed catheter. The initial daily doses of vitamin A in the two cases were 170 and 250 I. U. vitamin A/g body weight.

A second group of two animals with initial body weights of 238 and 141 grams were given a scorbutic diet consisting of water and bread.

A third group of two animals with initial body weights of 300 and 132 g were given 50,000 I. U. vitamin A daily in addition to the scorbutic diet, in the form of the same whale liver oil as given to the first group. The initial daily dose of vitamin A in the two animals was thus: 170 and 385 I. U. vit. A/g body weight daily.

a) The Control.

Guinea pig no. 18: The average daily weight increase was 4.5 grams (see graph no. 21). Throughout the entire experiment, — 38 days — the control animal was perfectly normal and free of any symptoms. It was killed in light ether anesthesia and examined in the usual manner after blood samples had been collected. The hemoglobin was 93 %. The ascorbic acid content of the serum was 0.18 mg/100 ml serum. The vitamin A content of the serum was 0.0 I. U. vit. A/g serum. The ascorbic acid content of the liver was 3.7 mg/100 g as determined by the 2,6-dichlorophenol-indophenol method.

By postmortem examination, normal organs were found except for several abscesses in the right kidney. By microscopical examination of the liver, kidneys, pancreas, spleen and testes no pathological findings were made. By sudan III staining of the liver, no sudanophil droplets were found, while by sudan III staining of the adrenals, deposits of sudanophil droplets were seen throughout the adrenal cortex .

The ash content of the femur was 57.5 %, calculated on a dry basis; the calcium and phosphorus contents of the ash were 34.1 % and 18.6 % respectively.

b) Basal Diet + Excess of Vitamin A.

In the group receiving excess of vitamin A in addition to the usual adequate basal diet, the following observations were made:

Guinea pig no. 18: The initial body weight was 296.5 grams, and at the end of the experiment (38 days) it was 370 grams, the average daily weight increase being 1.9 grams. The daily dose of vitamin A per gram body weight was from 170 to 130 I. U./g. The weight graph is shown on page 156.

In this animal no obvious clinical symptoms were observed. It was killed and examined at the end of 38 days, one hour after the last dose of vitamin A had been given. The hemoglobin was 85 %. The ascorbic acid content of the serum was 0.00 mg/100 ml (as against 0.18 for the control animal), and the vitamin A content of the serum was 3 I. U. vit. A/g serum. The ascorbic acid content of the liver was 0.3 mg/100 g (as against 3.7 for the control), as determined by the 2,6-dichlorophenol-indophenol method.

X-ray examination at the conclusion of the experiment, showed similar changes of the long bones as described for the other hypervitaminotic guinea pigs (see page 148), but no fractures.

By postmortem examination, considerable hemorrhages were observed in the muscles of the hind legs as well as of the fore legs, but no subcutaneous hemorrhages. There was marked visceral hyperemia. Both adrenals appeared enlarged, and accessory adrenals were observed on the right side. Otherwise no pathological findings were made.

Microscopical examination of the liver, kidney, adrenal, pancreas, testis and the tibia revealed similar findings as described for the other hypervitaminotic guinea pigs. There was slight degeneration of some of the renal tubules. In the tibia the cartilage cells and bone spicules were irregularly arranged and in the connective tissue there were scattered hemorrhages. By sudan staining of the liver, a large number of sudanophil droplets were seen in and between the liver cells, and in the adrenals there were especially dense deposits of sudanophil droplets throughout the cortex. Otherwise no significant pathological findings were made.

The ash content of the femur was 60.3 % as calculated on a dry basis. The calcium and phosphorus contents of the ash were 32.6 % and 18.2 % respectively.

Guinea pig no. 19: The initial body weight was 196.5 grams, and at the end of the experiment (25 days) it was 176.0 grams. A moderate weight gain took place during the first 18 days, after which the weight fell rapidly, the average daily loss of weight being approximately 0.9 grams during the entire experiment. The daily dose of vitamin A per gram body weight was at the commencement of the experiment 250 I. U., and varied during the experiment between 280 and 200 I. U./g. The weight graph is shown on page 156.

On the 21st day, fracture was diagnosed on the left hind leg, and the following day both fore legs appeared paralysed. X-ray examination at the conclusion of the experiment revealed fracture of both radius and ulna of one of the fore legs, as well as the usual thinning of the bones and changes in the tibial metaphyses.

When moribund on the 25th day, the animal was killed and examined in the usual manner. The blood coagulated normally. The vitamin A content of the serum was 22 I. U./g. The vitamin A content of the liver extracted by acetone in Soxhlet's apparatus, and determined spectrographically, was 5,255 I. U./g (276,000 I. U. vit. A/g fat).

By postmortem examination large subcutaneous hemorrhages were found in both hind legs (both proximal and distal of the knee) as well as over the back and abdomen, at the shoulders and proximal ends of the fore legs. The muscles in these places were also blood infiltrated. Blood extravasations were found in the left elbow joint as well as in the right knee joint.

By microscopical examination degeneration of the renal tubules was seen, some of which were filled with a calcium-like substance. There were red blood cells in the space of Bowman's capsule, as well as scattered red blood cells outside the capillaries elsewhere in the kidney. By sudan staining of the liver, a few sudanophil droplets were seen in and between the liver cells, and in the adrenal only a few sudanophil droplets were seen in the cortex.

The ash content of the femur calculated on a dry basis was 52.2 %, and the calcium and phosphorus contents of the ash were 34.5 % and 18.5 % respectively.

c) Scorbutic Diet.

In the group receiving scorbutic diet the following observations were made:

Guinea pig no. 20. The initial body weight was 238 grams, and at the end of the experiment (38 days) it was 274 grams, the average daily weight increase being approximately 0.9 grams (see graph no. 22). Apart from the reduced weight increase, no clinical symptoms were observed. The animal was killed and examined in the usual manner. The blood coagulated normally. The hemoglobin was 81 %. The ascorbic acid content of the serum was 0.05 mg/100 ml, and the vitamin A content of the serum was 0.0 I. U./g. The ascorbic acid content of the liver was 0.2 mg/100 g.

By postmortem examination, marked hyperemia was observed as well as hemorrhages in the muscles of the hind legs, distal of the knees. By microscopical examination of the liver, kidney, adrenal, pancreas, intestine, and the tibia, marked hyperemia was found. Scattered red blood cells were seen outside the capillaries, and a few red blood cells were seen in the space of Bowman's capsule. There was slight degeneration of some of the renal tubules, the cells of the convoluted tubules showing swelling and granularity, and a large number of vacuoles were seen in the adrenal cortex. In the bones similar findings as described for the hypervitaminotic guinea pigs were found. By sudan III staining, no deposits of fat were seen in the liver, while the adrenal cortex was packed with sudanophil droplets, except for a narrow band just inside the capsule. Otherwise no pathological findings were made.

The ash content of the femur calculated on a dry basis was 49.4 %, and the calcium and phosphorus contents of the ash were 33.3 % and 19.7 % respectively.

Guinea pig no. 21: The initial body weight was 141 grams, and at the conclusion of the experiment it was 108 grams, the average daily loss of weight being 2.6 grams. During the first 8 days there was a slight weight gain, after which the weight fell rapidly until the 12th day, when the animal appeared very weak, and suffered from soreness of the eyes. The following day it died.

By postmortem examination slight loss of hair was observed on the back. There were scattered subcutaneous hemorrhages, as well as muscular hemorrhages in both hind legs, both proximal and distal of the knee joints. In the left knee joint free blood was found. The lungs were fiery red, and the bones were brittle

as previously described for hypervitaminotic guinea pigs. By microscopical examination similar findings were made in the same organs as described for the previous animal.

X-ray examination of the bones revealed fracture of the femur as well as thinning of the entire bone shafts and the cortical shadows.

The ash content of the femur calculated on a dry basis was 50.1 %, and the calcium and phosphorus contents of the ash were 30.9 % and 17.8 % respectively, as against 52.7 %, 32.4 %, and 16.9 % respectively in guinea pigs given vitamin C in addition to scorbutic diet.

d) Scorbutic Diet + Excess of Vitamin A.

In the final group given the scorbutic diet and excess of vitamin A in the form of the same whale liver oil concentrate as given to the first group, in amounts corresponding to 50,000 I. U. vit. A daily, the following observations were made:

Guinea pig no. 22: The initial weight of this animal was 300 grams. A rapid loss of weight was observed from the very beginning of the experiment until the animal died on the 16th day, at which time the weight was 162 grams, — the average daily loss of weight being 8.6 grams (see graph no. 23). The vitamin A dose per gram body weight was 170 I. U./g at the commencement, and approximately 300 I. U./g at the conclusion of the experiment.

On the 13th day of the experiment the general condition of the animal was poor. It suffered from marked alopecia, as well as considerable dyspnoea. The following day these symptoms became more pronounced, and both hind legs appeared painful and paralysed, and there was soreness and swelling of the eyes. On the 15th day it was moribund, and died the following day. X-ray examination revealed fracture of the tibia just distal of the epiphyseal line, as well as thinning of the cortex.

Postmortem examination revealed scattered subcutaneous hemorrhages, as well as muscular hemorrhages in the hind legs, and free blood in the knee joints. In the popliteal fossa there was a large hematoma on both sides. There was detachment of the proximal tibial epiphyses. There was also visceral hyperemia, and scattered hemorrhages in the intestines, and hemorrhages in and around the adrenals.

Microscopical examination of the kidneys revealed hyperemia and slight degeneration of some of the renal tubules, as well as erythrocytes in the space of Bowman's capsule. By sudan staining of the liver only slight deposits of sudanophil droplets were seen, while dense sudanophil deposits were seen in the adrenal cortex. In the pancreas and the heart no significant pathological findings were revealed, apart from hyperemia.

The ash content of the femur calculated on a dry basis was 53.1 %, and the calcium and phosphorus contents of the ash were 30.3 % and 16.5 % respectively.

Guinea pig no. 23: The second of the guinea pigs in this group died at the end of 13 days. The initial body weight was 132 grams, and during the entire experiment the daily loss of weight was approximately 1.6 grams. The vitamin A dose per gram body weight was 385 I. U./g at the commencement of the experiment and 430 I. U. at the conclusion of the experiment.

At the end of 11 days the animal looked unwell. The following day it looked weak and suffered from soreness around the eyes. It died the following day.

By postmortem examination, slight alopecia was observed. There were scattered subcutaneous hemorrhages, as well as muscular hemorrhages of both hind legs, both proximal and distal of the knee. In the actual knee joint free blood was

observed. Visceral hemorrhages were also observed. The lungs were fiery red, and there was clubbing of the costochondral junction.

Microscopical examination revealed marked hyperemia in the internal organs, large deposits of sudanophil droplets in and between the liver cells, and in the adrenal cortex. Sudanophil deposits were also observed in the adrenal medulla. Red blood cells were seen outside the capillaries in the kidney, and there was slight degeneration of some of the renal tubules. No pathological findings were made in the lungs or intestines apart from hyperemia. In the bones there was hyperemia and destruction of bone spicules. The ash content of the femur calculated on a dry basis was 42.2 %, and the calcium and phosphorus contents of the ash were 33.1 % and 19.5 % respectively.

X-ray examination revealed fracture of the proximal metaphyses of the tibiae.

From this experiment the similarity in the clinical picture, as well as the postmortem findings in hypervitaminotic and scorbutic guinea pigs appears evident. Furthermore, the same low content of ascorbic acid in the liver and in the serum was detected in both cases, and it was shown that similar gross doses of vitamin A proved more toxic to guinea pigs given a scorbutic diet, than to guinea pigs given the usual adequate basal diet.

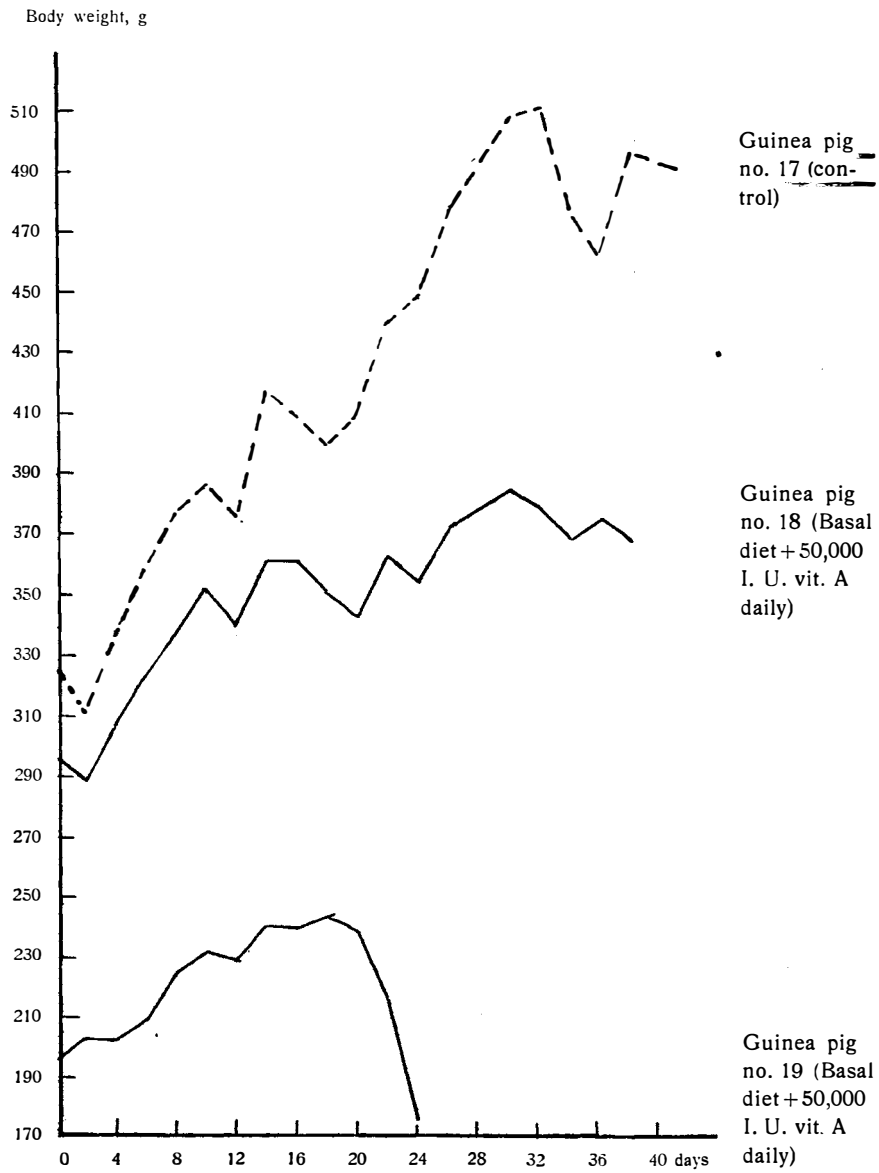
Table 72.

Body Weights and Vitamin A Dosage in Experiment 45.

Conditions of the experiment	Guinea pig no.	Initial body weight, g	I.U. vit. A/g body weight daily	Duration of experiment, days	Lethal effect	Weight gain, g/day, average
Basal adequate diet (control)	17	327	-	38	0	+ 4.5
Basal adequate diet + 50,000 I.U. vit. A daily	18	297	170—130	38	0	+ 1.9
	19	196	280—200	25	+	— 0.9
Scorbutic diet	20	238	-	38	0	+ 0.9
	21	140	-	13	+	— 2.6
Scorbutic diet + 50,000 I.U. vit. A daily	22	300	170—300	16	+	— 8.6
	23	132	385—430	13	+	— 1.6

4. *The Effect of Vitamin C in Hypervitaminosis A.*

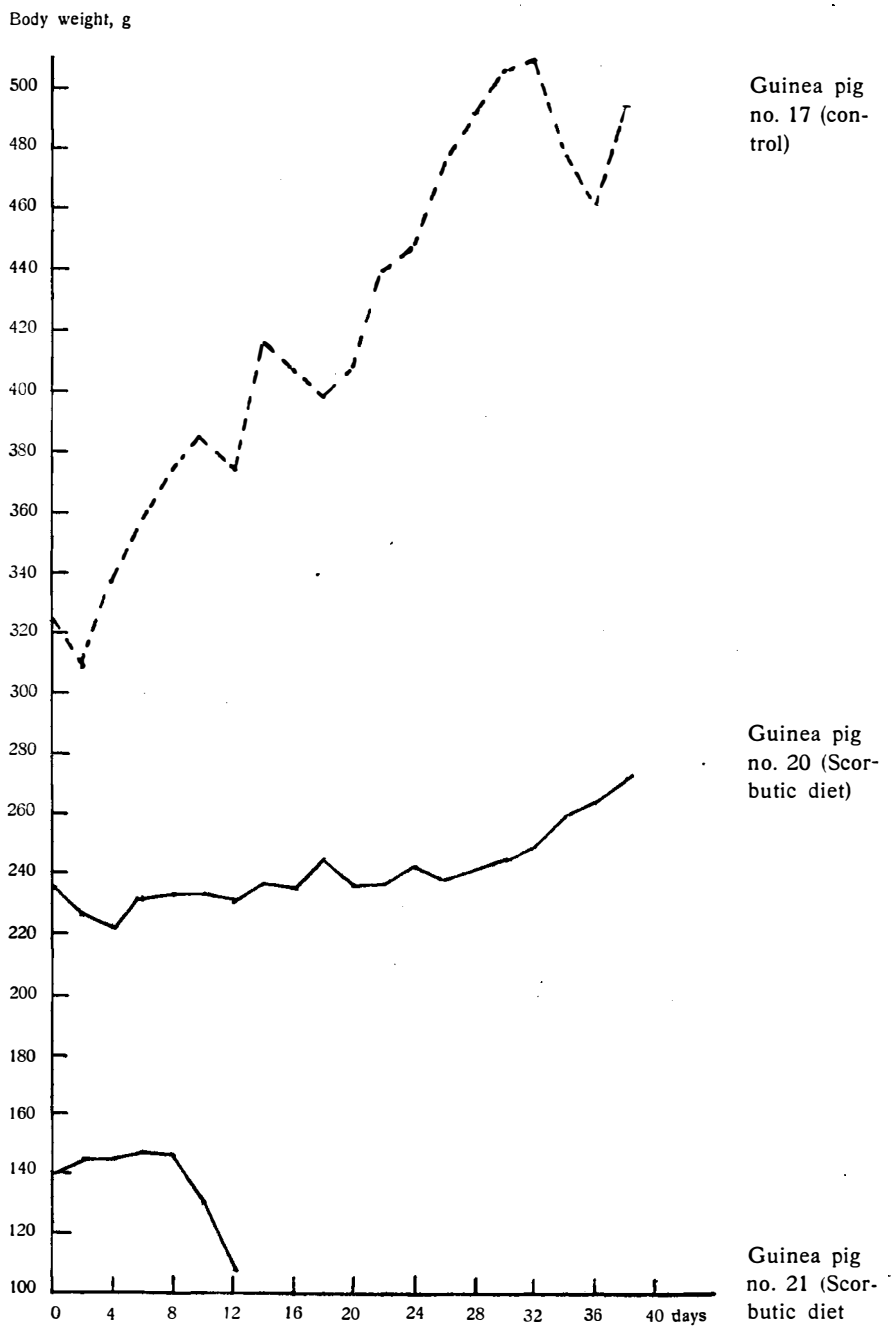
From the preliminary investigations, no definite protective effect was obtained against the injurious effect of excess of vitamin A in young guinea pigs by massive doses of vitamin C given by subcutaneous injection. It was therefore considered desirable to re-examine the effect of vitamin C given by subcutaneous injection in slightly older animals,



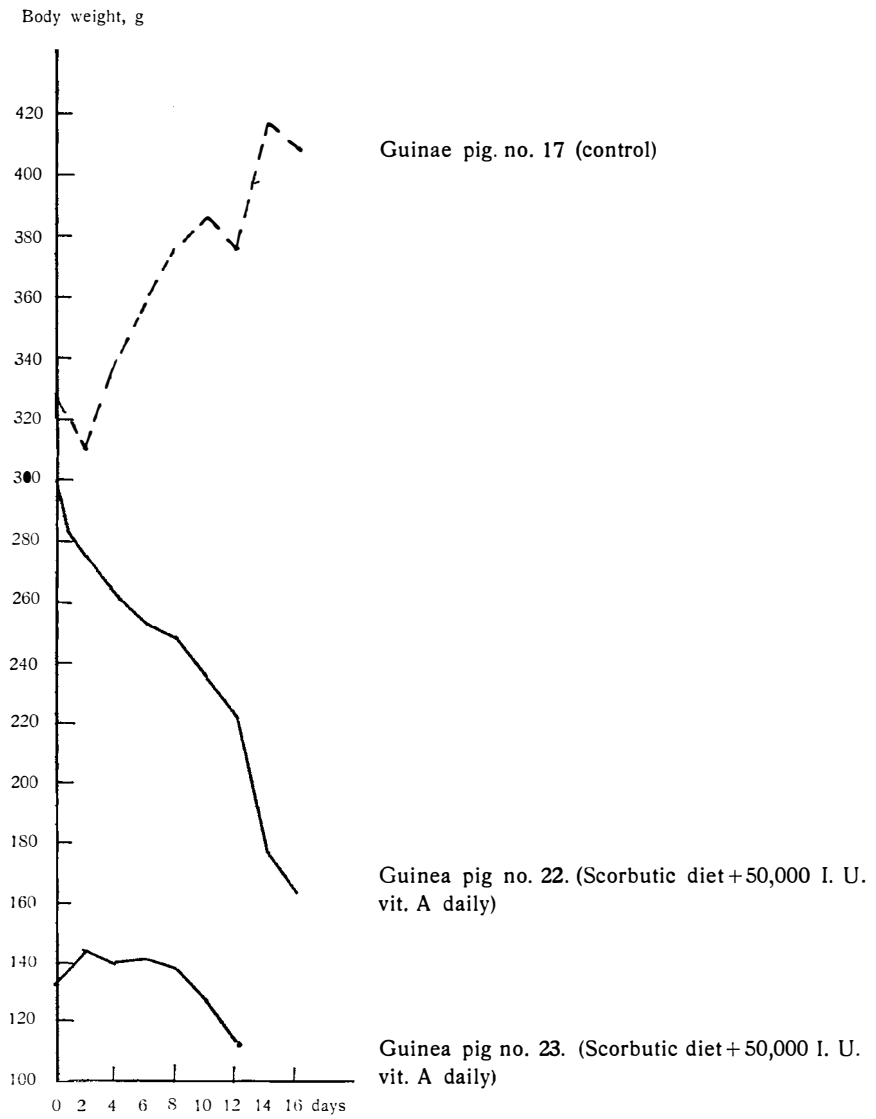
Graph No. 21. Weight graphs for guinea pigs given basal diet + excess of vitamin A, compared with the normal control (Experiment 45).

and to examine the effect of oral administration of the same massive doses of vitamin C in hypervitaminotic guinea pigs of varying ages.

In each of the following experiments the effect of massive doses of vitamin C in addition to excess of vitamin A was compared with the effect of excess of vitamin A alone, in guinea pigs with approximately the same initial body weight.



Graph No. 22. Weight graphs of guinea pigs given scorbutic diet, compared with normal control (Experiment 45).



Graph No. 23. Weight graphs of guinea pigs given scorbutic diet + excess of vitamin A, compared with normal control (Experiment 45).

Experiment 46.

In this experiment, two guinea pigs of the same litter were used, with initial body weights between 150 and 160 grams.

One of the animals (no. 24), received in addition to the usual adequate basal diet, vitamin A alcohol dissolved in peanut oil (640,000 I. U. vit. A/g) in amounts corresponding to 60,000 I. U. daily (400 I. U. vit. A/g

body weight). The second animal (no. 25) received in addition to this 50 mg ascorbic acid daily by subcutaneous injection.

One day after the beginning of the experiment the first mentioned animal showed symptoms of unwell-being. It was apatic, showed lack of activity and the pelt looked scruffy. Two days later, soreness around the eyes and nose was observed. On the 5th day it limped, and suffered from dyspnoea. The following day it died, and by postmortem examination, general alopecia was observed. Both eyes were practically closed and covered by pus. A subcutaneous hemorrhage was observed on the medial side of the right thigh, as well as other scattered subcutaneous hemorrhages. In the lungs there were signs indicating pneumonia. The liver appeared fatty on the cut surface. The kidney appeared speckled on the surface, and small hemorrhages were seen in the adrenals.

By microscopical examination of the liver, kidney, adrenal, pancreas and intestine, marked hyperemia was observed. By sudan staining of the liver a large number of sudanophil droplets were seen, particularly between the liver cells. The adrenal cortex contained a large number of vacuoles and by sudan staining large deposits of sudanophil droplets were seen in the cortex at the border of the medulla, and in the zona glomerulosa. Some sudanophil droplets were also seen in the medulla. Hyperemia was also observed in the bones (tibia) and the structure of the bone was irregular with enlarged Howship's lacunae. In the teeth there was marked hyperemia in the pulp as well as scattered hemorrhages, and a number of vacuoles. The odontoblasts were irregularly arranged. In the gingiva signs of inflammation with infiltration of lymphocytes were seen in patches.

The ash content in the femurs was 47.4 % calculated on a dry basis. The calcium content of the ash was 31.9 %, and the phosphorus content 17.9 %.

On the third day of the experiment the second guinea pig (no. 25), given ascorbic acid in addition to excess of vitamin A, suffered from soreness around the eyes and nose. Two days later limping was observed. On the 7th day it died, and by postmortem examination it was found to suffer from pneumonia.

The ash content of the femur calculated on a dry basis was 47.3 %, and the calcium and phosphorus contents of the ash were 29.0 % and 17.7 % respectively.

X-ray examination of the long bones showed similar findings in both animals as described on page 148.

From this experiment it appears that 50 mg vitamin C daily, given by subcutaneous injection offered no protection against the injurious effect of 400 I. U. vit. A/g body weight in young guinea pigs, which is in agreement with the observations made during the preliminary investigations.

Experiment 47.

The purpose of this experiment was to examine whether oral administration of massive doses of vitamin C offered any protection against the injurious effect of excess of vitamin A in young guinea pigs, with initial body weights of 96—120 grams.

One guinea pig (no. 30), was used as a control, and was given the usual adequate basal diet only. A group of two animals received two drops of a highly concentrated source of vitamin A, in addition to the basal diet, i. e. vitamin A alcohol dissolved in a minimum of peanut oil, so as

to yield 640,000 I. U. vit. A/g. This dose corresponded to a daily vitamin A intake of 35,000 I. U. vit. A. A second group of two animals received 50 mg ascorbic acid daily in addition to the same basal diet and the same dose of vitamin A. Otherwise the conditions of the experiment were identical in the two groups. In the course of the experiment, the following observations were made:

a) Excess of Vitamin A.

Two days after the beginning of the experiment, one of the guinea pigs (no. 26) receiving excess of vitamin A (350—465 I. U./g body weight daily) appeared weak and scruffy. The following day it limped on the right hind leg, and showed eye symptoms. On the 4th day of the experiment it died, while none of the other animals appeared affected. There was a marked loss of weight, — approximately 5 grams daily, while the control animal gained an average of approximately 6 grams.

X-ray examination of the long bones revealed abnormally thin cortex and lines of dense calcification at the epiphyseal border of the tibial metaphyses. The mineral content of the bones is given in table 76.

By postmortem examination, alopecia as well as soreness around the eyes were observed. There was marked hyperemia, but no macroscopical hemorrhages. The adrenals appeared enlarged, and the bones were brittle.

Microscopical examination revealed marked hyperemia in the bones, as well as in the internal organs, such as the pancreas, adrenals, and kidneys. In the kidneys there were a few red blood cells in the space of Bowman's capsule, and degeneration of some of the renal tubules, some of which contained deposits of a calcium-like substance. By sudan staining of the liver a few sudanophil droplets were seen. One of the ribs showed fracture with hemorrhage.

The second guinea pig in this group (no. 27) receiving the same dose of vitamin A (35,000 I. U. daily, corresponding to 300 I. U./g body weight) appeared weak and scruffy, with soreness and swelling of the right eye on the 6th day. The following day it was practically moribund, and there was prolapse of the rectum. On the 8th day it died. Also in this case a marked loss of weight was observed, — an average of approximately 4 grams daily.

X-ray examination of the long bones revealed similar changes as described for the previous guinea pig. The mineral content of the bones is given in table 76.

By postmortem examination, hyperemia and small subcutaneous hemorrhages were found, as well as hemorrhages in the muscles of the hind legs. There were no fractures.

Microscopical examination showed similar findings as described for the previous guinea pig. The degeneration of the renal tubules was more pronounced, however, and the compact bone in the tibia was very thin.

b) *Excess of Vitamin A + Vitamin C.*

In the two guinea pigs given the same dose of vitamin A (35,000 I. U. vit. A daily)with an addition of 50 mg ascorbic acid daily, the following observations were made:

One of them (no. 28), with an initial body weight of 120 grams, consuming 290—390 I. U. vit. A/g body weight, showed a considerable loss of weight from the third day. On the 8th day it appeared very weak. Two days later it was moribund, and died the following day, the average daily loss of weight being 3.3 grams.

X-ray examination of the long bones showed abnormally thin cortex and lines of dense calcification at the epiphyseal border of the tibial metaphyses, as well as fractures of the proximal tibial metaphyses (see ill. 23). The mineral content of the bones is given in table 76.

By postmortem examination, slight alopecia and soreness was observed around the mouth. There were numerous small subcutaneous hemorrhages, and also free blood in and around the knee joints, as well as in the muscles of the legs, particularly in between the muscle sheaths. There were also scattered visceral hemorrhages.

Microscopical examination of the liver, kidney, adrenal, pancreas and heart showed marked hyperemia, and in some places scattered red blood cells outside the capillaries in the liver and kidney. By sudan staining of the liver, very few sudanophil droplets were seen. In the bones (tibia) there was evidence of increased osteoclastic activity. In some places near the proximal tibial epiphyses, the compact bone had completely disappeared, and was substituted by connective tissue, in which scattered red blood cells were seen.

The second guinea pig in this group (no. 29) had an initial body weight of 105 grams, and the vitamin A consumption was approximately 350 I. U. vit. A/g body weight. The weight fell from the very beginning of the experiment, the average daily loss of weight being 4.0 grams. At the end of 6 days the general condition of the animal was very poor, and the following day it died.

By postmortem examination, no significant pathological findings were made by the external inspection. There were subcutaneous hemorrhages however in the right inguinal region, and in the left axilla, and a large hemorrhage in the muscles of the left hind leg. There were also hemorrhages in the pericardium, lungs and pancreas, as well as free blood in the left knee.

Microscopical examination of the lungs showed very marked hyperemia, free blood in the alveoli and in the lung tissue, which showed atelectasis in some areas. There was desquamation of the epethelial cells in the alveoli which contained a large number of macrophages and a few lymphocytes. Marked hyperemia was also observed in the liver, kidneys, adrenals, pancreas and intestines. In the tibia there was a fracture with periostal hemorrhage. The cartilage cells were irregularly arranged in some places, and the structure of the bone appeared chaotic.

The mineral content of the bones is given in table 76.

Table 73.

Weight Gain in Guinea Pigs Given Excess of Vitamin A, Compared With Guinea Pigs given Excess of Vitamin A + Vitamin C.

Conditions of the experiment	Guinea pig no.	Initial body weight, g	I. U. vit. A/g body weight, daily	Time of survival, days	Weight gain, g/day
Basal diet (control)	30	108	-	-	+ 6 0
Basal diet + 2 drops of vit. A concentrate daily (35,000 I. U.)	26	96	350—465	4	— 5.0
	27	114	300	8	— 4.0
Basal diet + 2 drops of vit. A concentrate (35,000 I. U.) + 50 mg ascorbic acid daily	28	120	290—390	11	— 3.3
	29	105	350	7	— 4.0

From this experiment it may be concluded that oral administration of 50 mg ascorbic acid daily offered no protection against the injurious effect of 300—400 I. U. vit. A/gram body weight daily in young guinea pigs.

Experiment 48.

In this experiment, the effect of oral administration of massive doses of vitamin C in addition to excess of vitamin A was studied in slightly older guinea pigs, when observed during a comparatively long period of time.

For this purpose 8 guinea pigs were used, with initial body weights between 135 and 330 grams.

The animals were divided into three groups. One group consisting of two animals, was used as control, and received the ordinary adequate basal diet.

A second group, consisting of 4 animals, received in addition to the same basal diet, whale liver oil concentrate in amounts corresponding to 50,000 I. U. vit. A daily, given by a specially constructed catheter attached to a small syringe, which allowed an accurate dosage.

A third group consisting of two animals received in addition to the same diet and dosage given to the second group, 50 mg ascorbic acid daily, given by dropping pipet.

In order to maintain identical experimental conditions, the control guinea pigs were given peanut oil in the same quantities as the whale liver oil given to the second and third groups.

The control animals remained healthy and free of any symptoms throughout the experiment, which lasted 65 days. The average daily weight increase was approximately 5.0 and 5.4 grams respectively in the two animals. The individual weight graphs of the guinea pigs in this experiment are given in graph no. 24.

a) Excess of Vitamin A.

Throughout the experiment 50,000 I. U. vit. A was given daily in addition to the usual adequate basal diet. Great care was taken that the entire dose was swallowed and to avoid any oil being smeared on the skin of the animal.

In one of the guinea pigs (no. 31), the daily intake of vitamin A/gram body weight at the commencement of the experiment was approximately 230 I. U. The maximum intake was 250, and the minimum 156 I. U. vit. A/g body weight daily. The average daily dose throughout the entire experiment was 178 I. U./g body weight.

On this dose of vitamin A the weight increased by 5.0 grams daily (normal) during the first 20 days, after which the weight remained practically constant during a further 24 days, at the end of which time the weight fell rapidly until the animal died on the 58th day, when the weight was practically the same as at the beginning of the experiment.

Apart from the absence of normal weight gain, no clinical symptoms were observed until the animal showed peeling of the epidermis on the feet on the

43rd day (see ill. 8). The skin could be peeled off in large flakes. This peeling persisted approximately a week.

On the 52nd day, limping was observed as well as alopecia and soreness around the eyes. These symptoms persisted until the animal became moribund on the 57th day. The following day death occurred, preceded by cramps. The cramps were both clonic and tonic, and involved all four extremities.

When moribund, blood samples were collected for the determination of the vitamin A content, which was 10 I. U./g serum, 24 hours after the last dose of vitamin A had been given.

By postmortem examination marked alopecia over the whole body was observed. There were fractures of one of the hind legs as well as both fore legs. There was marked hyperemia, and small subcutaneous hemorrhages over the left shoulder, as well as muscular hemorrhages in both thighs. There was marked hyperemia in the intestines, and the adrenals appeared considerably enlarged. The kidneys appeared swollen. The heart was large and flabby. Free blood was observed in the pleura cavity.

Histologically, arterial hyperemia was observed in the liver, adrenals, kidneys, pancreas, testes and the bones. There was slight degeneration of the renal tubules, and in the liver stained by sudan III, dense deposits of large sudanophil droplets were seen both in and between the liver cells. In the adrenal, moderate sudanophil deposits were observed throughout the cortex. The costochondral junction appeared swollen, there was marked hyperemia, irregular bone structure with signs of increased osteoclastic activity, and the compact bone was abnormally thin.

In the second guinea pig in this group (no. 32), the initial dose of vitamin A per gram body weight was approximately 350 I. U./g, the average dose throughout the experiment being 400 I. U./g body weight daily. At the end of 5 days it appeared weak, and three days later it died. X-ray examination revealed fracture of the femur. There was marked loss of weight from the beginning of the experiment, approximately 5 grams daily. By postmortem examination free blood was found in both knee joints. There were large muscular hemorrhages involving all the muscles of the left thigh. The adrenals appeared enlarged. In both lungs there were signs indicating pneumonia. The bones were very brittle, as previously described for the other hypervitaminotic guinea pigs (see page 141).

Microscopical examination of the liver, kidney, adrenal and pancreas revealed hyperemia, erythrocytes in the space of Bowman's capsule in the kidney, as well as degeneration of some of the renal tubules. In the pancreas, necrosis was observed in some places. By sudan staining very few sudanophil droplets were seen in the liver, while sudanophil droplets were evenly distributed throughout the adrenal cortex.

The ash content of the femur calculated on a dry basis was 46.8 %, and the calcium and phosphorus contents of the ash were 35.5 % and 17.3 % respectively.

In the third guinea pig in this group (no. 33), the initial daily dose of vitamin A/gram body weight was approximately 215 I. U./g. Towards the end of the experiment the dose was approximately 120 I. U. vit. A/g, the average dose during the whole experiment being 140 I. U./g daily. On this dose the average daily weight increase was approximately 2.2 grams.

Apart from the absence of the normal weight gain, no clinical symptoms were observed until peeling of the epidermis occurred on the feet on the 58th day of the experiment, at which time soreness around the papillae mammae was observed. This peeling persisted until the animal was killed on the 65th day, at which time there was also alopecia. Otherwise no symptoms were detected.

The animal was examined in the usual manner. The hemoglobin was 70 %, the ascorbic acid content of the serum was 0.00 mg/100 ml. By postmortem

examination no significant pathological findings were made apart from clubbing of the costochondral junction and a fatty cut surface of the liver. The kidneys and adrenals appeared enlarged.

Histologically, hyperemia was observed in the liver, adrenals, kidneys, pancreas and the bones. There was slight degeneration of some of the renal tubules. By sudan III staining, massive deposits of sudanophil droplets were observed in the liver both in and between the liver cells, particularly around the central vein, and throughout the adrenal cortex. In the costochondral junction, similar findings were made as described in guinea pig no. 31, and in addition to this, old hemorrhages were found in the sorrounding muscles.

In the fourth guinea pig (no. 34), the initial daily dose of vitamin A per gram body weight was approximately 250 I. U., varying between 160 and 280 I. U./g body weight daily, the average dose being 195 I. U./g during the whole experiment.

During the first 45 days, there was a moderate average weight increase of approximately 2.5 grams daily, followed by a rapid loss of weight until the animal died on the 61st day of the experiment, at which time the weight was 30 grams less than at the beginning of the experiment. The average loss of weight was thus approximately 0.5 g daily.

Also in this case the animal remained free of clinical symptoms, apart from the absence of the normal weight gain, until the 43rd day, when peeling of the epidermis on the left foot occurred. This peeling persisted until the animal died 18 days later, at which time there was peeling on both feet. On the 49th day there was soreness of both eyes, and the general condition of the animal was poor with marked loss of weight and increasing weakness. Two days later fracture of the left fore leg was diagnosed. On the 56th day alopecia was observed, particularly around the eyes and anus. The general condition gradually became worse until the animal became moribund and died.

By postmortem examination immediately after death, fracture of the proximal end of the left tibia was also observed. There were large hemorrhages around the fractures, and free blood in the left knee joint. There was marked hyperemia, and the liver had a nutmeg appearance. The pancreas and lungs were fiery red. The kidneys appeared moderately enlarged and speckled, and the adrenals considerably enlarged. The heart was large and flabby, the costochondral junction appeared clubbed.

Histologically, marked hyperemia was observed in the liver, adrenal, kidney, pancreas and the bones. In the kidney, there was marked degeneration of some of the tubules, which were filled with amorphous masses and a calcium-like substance. Erythrocytes were observed in some of the tubular lumen, and in the space of Bowman's capsule. In the liver stained by sudan III, massive deposits of sudanophil droplets were seen both in and between the liver cells (in the Kupffer cells), throughout the organ, particularly dense in the areas around the central veins. Massive sudanophil deposits were also seen throughout the adrenal cortex. The costochondral junction was widened, and appeared deformed. There were periostal and subperiostal hemorrhages, irregular calcification, and great irregularity of bone structure with destruction of bone spicules. In the tibia, the bone structure was chaotically arranged. In some places the compact bone had disappeared, and there were numerous hemorrhages in the bone, periost and sorrounding muscle.

X-ray examination of the long bones at the conclusion of the experiment, revealed abnormally thin bone shafts and cortex, and densely calcified lines at the border of the epiphyses.

b) Excess of Vitamin A + Vitamin C.

The two guinea pigs in this group received 50 mg ascorbic acid daily by dropping pipet in addition to the usual adequate basal diet and the same doses of vitamin A as given to the previous group.

One of these animals (no. 35) survived during a period of 10 days, during which time the weight remained unchanged. The average daily vitamin A intake per gram body weight was 380 I. U./g.

At the end of 8 days it appeared weak. On the 10th day it was moribund, and suffered from diarrhea and dyspnoea. It gradually became worse in the course of the day, and finally suffered from attacks of cramps, lasting from seconds up to several minutes. The pauses between each attack lasted a few minutes.

By postmortem examination large dilated intestines were found. There were muscular hemorrhages in all four extremities, as well as scattered small subcutaneous hemorrhages. There was marked hyperemia. The pancreas and the lungs were fiery red, but no evidence of pneumonia was detected. The bones were very brittle.

Microscopical examination revealed hyperemia in the liver, kidney, adrenal, pancreas and heart. In the kidneys red blood cells were seen in the space of Bowman's capsule. By sudan staining very few sudanophil droplets were seen in the liver, while moderate deposits of sudanophil droplets were detected in the adrenal cortex.

The ash content of the femur calculated on a dry basis was 51.2 %, and the calcium and phosphorus contents of the ash were 35.8 % and 18.0 % respectively.

The second guinea pig (no. 36) in this group had a daily intake of 240 I. U. vitamin A per gram body weight at the beginning of the experiment, and towards the conclusion of the experiment it was 115 I. U./g, the average daily vitamin A intake throughout the entire experiment being 175 I. U./g body weight.

The average daily weight gain was 3.6 grams. The animal remained free of any symptoms throughout the experiment, except for the slightly reduced weight gain.

On the 65th day of the experiment it was killed and examined in the usual manner, at which time it appeared quite normal. By postmortem examination no pathological findings were revealed. There were no hemorrhages and no fractures, and the bones appeared normally developed.

Histologically hyperemia was detected in the liver, adrenal, kidney, pancreas, testis and bones. In the kidney there was degeneration of some of the tubules, and some red blood cells were seen in the space of Bowman's capsule. In the liver, stained by sudan III, very dense deposits of sudanophil droplets were observed throughout the organ, both in and between the liver cells, particularly in the Kupffer cells. Very dense sudanophil deposits were also seen in the adrenal cortex, the peripheral half of which contained a continuous broad band of brightly coloured sudanophil droplets. Considerable deposits of sudanophil droplets were also seen in the medulla. There was a marked widening of the costochondral junction, irregular bone structure, and formation of connective tissue, where scattered red blood cells were seen outside the capillaries.

The hemoglobin was 89 %, and the ascorbic acid content of the serum was 0.42 mg/100 ml. The total base was 156.5 milliequivalents/liter, the calcium content was 10.7 mg/100 ml in the serum, and the vitamin A content of the serum was 0.0 I. U. (24 hours after the last dose of vitamin A).

Table 74.

Body Weights and Vitamin A Dosage in Experiment 48.

Conditions of experiment	Guinea pig no.	Initial body weight, g	Time of survival, days	I.U. vit. A/g body weight daily	Lethal effect	Weight increase g/day
Basal diet (control)	37	142	killed 65th	-	0	+ 5.4
	38	329	killed 65th	-	0	+ 5.0
Basal diet + excess of vitamin A (50,000 I.U. daily)	31	207	58	250—156	+	0.0
	32	143	8	350	+	— 5.0
	33	238	killed 65th	215—120	0	+ 2.2
	34	195	61	280—160	+	— 0.5
Basal diet + excess of vitamin A (50,000 I.U. daily) + 50 mg asc. acid daily	35	134	10	380	+	0.0
	36	208	killed 65th	240—115	0	+ 3.6

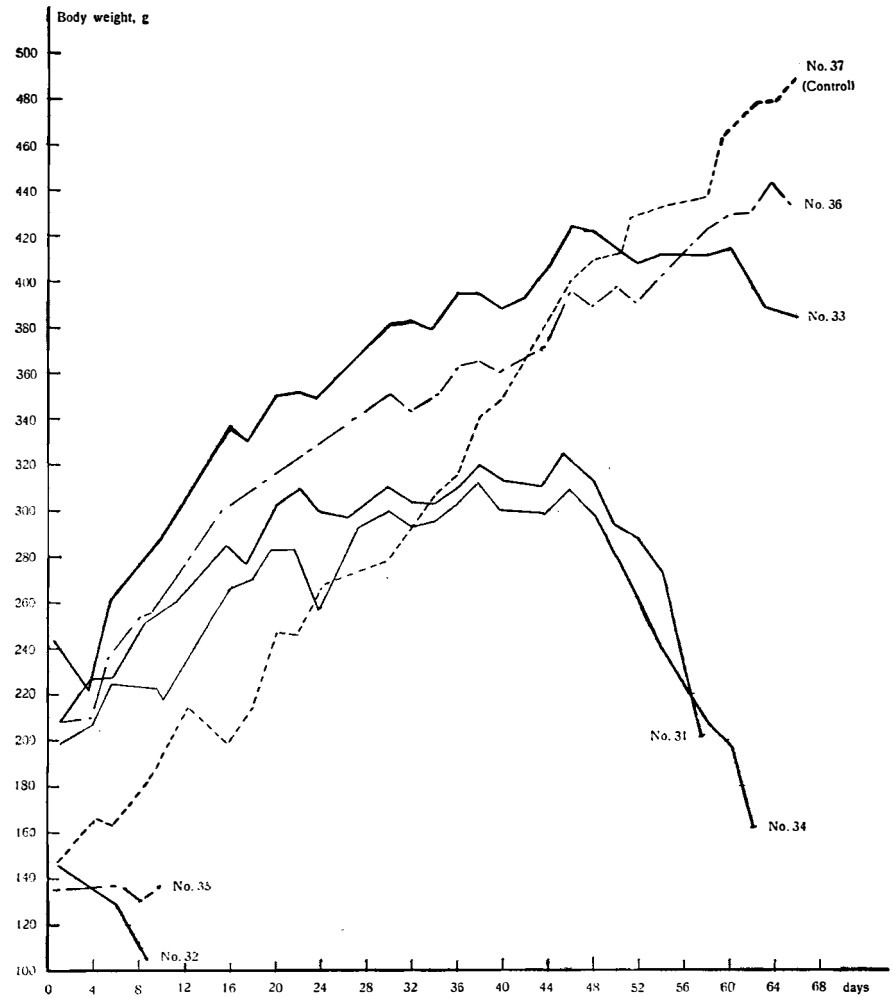
From this experiment it may be concluded that oral administration of 50 mg ascorbic acid daily offered no protection against the injurious effect of 380 I. U. vit. A/g body weight in young guinea pigs, which is in agreement with the observation made in the previous experiment.

The same dose of vitamin C appeared, however, to have some beneficial effect against 115—240 I. U. vit. A/g body weight daily, as judged by clinical examination.

Summary of Results. Excess of vitamin A was found to be toxic to guinea pigs when given in amounts over 50 I. U. vit. A/g body weight daily. Both oral administration and subcutaneous injection of massive doses proved toxic. The toxic effect was identical whether the vitamin A was given in the form of highly purified preparations, or in the form of whale liver oil concentrate, and increased with increasing doses of vitamin A. Thus approximately 50—100 I. U. vit. A/g body weight daily caused toxic symptoms, while doses between 110—550 in all cases except two proved lethal within 4 to 61 days. The manifestation of clinical symptoms in hypervitaminotic guinea pigs is given in table 75.

Absence of normal weight gain was one of the most constant findings and appeared in all cases given excess of vitamin A in addition to the usual basal diet. On the whole it was found that the higher the excess of vitamin A the greater was the reduction of the weight increase.

The severity of the symptoms was generally found to vary with the given doses of vitamin A. Thus 50—90 I. U. vit. A/g body weight caused only slight symptoms, apart from the absence of normal weight gain, while doses from 300—500 I. U./g body weight caused pronounced symptoms, and invariably proved fatal.



Graph No. 24. Weight graphs of guinea pigs nos. 31—34 given basal diet + excess of vitamin A (50,000 I.U. daily), and of guinea pigs nos. 35—36 given basal diet + excess of vitamin A (50,000 I.U. daily) + 50 mg asc. acid daily, compared with the normal control guinea pig. no. 37. (Experiment 48.)

Following massive doses of vitamin A, the animal was usually found to look unwell, showed lack of activity, appeared weak and the pelt looked scruffy. Alopecia was observed in most cases. Muscular weakness was pronounced in 6 animals, and eye symptoms, similar to those observed in hypervitaminotic rats, occurred in 9 out of 17 animals. In two cases the animal lost the use of its hind legs, which appeared to be paralysed. Fractures of the legs occurred in 5 animals out of 17. In one case distinct pains in the hind legs were observed, without any fracture being detected. In this case the pain was found probably to be due to

Table 75. *Manifestation of Clinical Symptoms, and Postmortem*

Conditions of experiment	Guinea pig no.	Sex	Initial body weight, g	Vit. A dose		Survival, days	Lethal effect	Reduced weight gain	Weakness	Lack of activity	Scruffiness	Alopecia
				I. U. vit. A/day	I. U. vit. A/g body weight/day							
Basal diet + excess of vit. A	2	♂	92	50,000	550	7	+	+	-	-	+	+
	8	-	55	10,000	180	6	+	+	+	-	+	-
	9	♂	74	10,000	130	12	+	+	+	-	+	+
	11	♂	108	15,000	90	-	-	+	-	-	-	-
	12	♂	254	10,000	55	24	+	+	-	-	-	+
	13	♂	134	50,000	370	12	+	+	-	+	+	+
	14	♂	101	17,500	200	9	+	+	-	-	-	+
	15	♂	156	17,500	120	17	+	+	-	-	+	+
	18	♂	297	50,000	150	-	-	+	-	-	-	-
	19	♂	197	50,000	240	25	+	+	-	-	-	-
	24	♂	152	60,000	400	6	+	+	-	+	+	+
	26	♀	96	35,000	400	4	+	+	+	-	+	+
	27	♂	114	35,000	300	8	+	+	+	-	+	-
	31	♀	207	50,000	200	58	+	+	-	-	-	+
	32	♀	143	50,000	350	8	+	+	+	-	-	-
	33	♂	238	50,000	180		-	+	-	-	-	+
	34	♀	195	50,000	210	61	+	+	+	-	-	+
Basal diet + excess of vit. A + vit. C (50 mg/day)	3	♂	88	50,000	550	8	+	+	-	-	-	-
	25	-	159	60,000	400	7	+	+	-	-	-	-
	28	♀	120	35,000	340	11	+	+	+	-	-	+
	29	♂	105	35,000	350	7	+	+	-	-	-	-
	35	♀	134	50,000	380	10	+	+	+	-	-	-
	36	♂	208	50,000	175	-	-	+	-	-	-	-
Scorbutic diet	4	♀	91		-	7	+	+	-	-	+	+
	20	♀	238	-	-	-	-	+	-	-	-	-
	21	♀	140	-	-	13	+	+	+	-	-	+
Scorbutic diet + excess of vit. A	5	-	118	50,000	415	7	+	+	-	-	-	-
	22	♀	300	50,000	235	16	+	+	-	-	-	+
	23	♂	132	50,000	400	13	+	+	+	-	-	+

[1] Positive findings are indicated by: +.

muscular hemorrhage. Cramps in the legs were observed in two animals just prior to death, the cause of which was unknown. In two cases there was disunion of the proximal tibial epiphyses. In three cases peeling of the epidermis on the feet was observed. This symptom occurred late — from the 43rd to the 58th day of the experiment.

By postmortem examination the most constant findings were hyperemia and hemorrhages, which occurred in practically all cases (see table 75). The hemorrhages were both subcutaneous, muscular and visceral. Muscular hemorrhages in the legs occurred in 8 out of 17 cases, and free blood in the knee joints was detected in 7 cases. In 7 cases the

Findings in Guinea Pigs given Excess of Vit. A.[1]

Soreness of skin	Peeling of skin	Eye symptoms	Pains in limbs	Limping	Disunion of epiphyses	„Paralysis" of legs	Cramps	Positive X-ray findings	Fracture	Hyperemia	Subcutaneous hemorrhages	Muscular hemorrhages	Visceral hemorrhages	Free blood in knee joint	Enlarged adrenal	Clubbing of costochondral junction	Red blood cells outside capillaries	Red blood cells in space of Bowman's capsule	Degeneration of renal tubules
-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-
-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+
+	+	+	-	-	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+
+	+	+	-	-	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+
+	+	+	-	+	-	-	-	+	-	+	+	+	+	-	+	+	-	-	(+)
+	+	+	-	+	-	-	-	+	+	+	+	+	-	+	+	-	+	+	+
+	+	+	-	-	-	-	-	+	+	+	-	+	+	+	+	-	-	-	-
+	+	+	-	-	-	-	+	-	-	+	+	+	+	+	+	-	-	+	+
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+	+	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-
-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	-	-	+	+	+
-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+	+	-
+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	-	-	+	+	-
-	-	-	-	+	-	+	+	-	+	+	+	+	+	-	-	-	+	-	+
+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+
+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-

adrenals appeared enlarged. In several cases the lungs and pancreas were fiery red. Clubbing of the costochondral junction was observed in three cases.

By microscopical examination, apart from hyperemia, — degeneration of the renal tubules, erythrocytes in the space of Bowman's capsule; and scattered red blood cells outside the capillaries in the internal organs, where the most constant findings (see table 75). Large deposits of sudanophil droplets were detected in the liver and adrenal cortex.

In the long bones, which were brittle, there was usually hyperemia, and irregularity of the cartilage cells. There was great irregularity in

the structure of the compact bone. In several cases periostal hemorrhages were detected. In all cases there was irregular calcification and large Howship's lacunae with evidence of increased osteoclastic activity. In several cases there was destruction of bone spicules, and scattered red blood cells were seen in the connective tissue.

In the teeth there was marked hyperemia in the pulp, where vacuoles and scattered hemorrhages were seen. There was complete irregularity of the odontoblasts, which in some places were represented by heaps of cells chaotically arranged.

Roentgenograms of the long bones revealed similar roentgenographical bone changes as observed in the hypervitaminotic rats, — i. e. abnormally thin bone shafts and cortex, and densely calcified lines at the epiphyseal borders of the tibial metaphyses. In some cases there was disunion of the epiphyses, and the fractures usually occurred near the end of the long bones.

In some cases the ash contents of the bones were considerably reduced in hypervitaminotic guinea pigs and considerable individual variations were observed. No significant difference was found in the average mineral content of the ash, however, as compared with the control animals (see table 76).

The hemoglobin was normal in all cases, except one. The ascorbic acid content of the blood was determined in 4 hypervitaminotic guinea pigs, and was in three cases 0.00 mg/100 ml, and in the 4th case 0.14, as against 0.18—0.20 mg/100 ml for the normal control animals. The vitamin C content of the liver in the hypervitaminotic animals was considerably less than in the control (0.3 as against 3.7 mg/100 g). The vitamin A content of the serum was between 3 and 22 I. U. vit. A/g in the hypervitaminotic guinea pigs.

The condition of hypervitaminosis A in guinea pigs resembled to a great extent the condition of scurvy, both with regard to the clinical picture and the postmortem findings. Thus the absence of normal weight gain was observed in both cases. The bone abnormalities appeared to be similar, as judged by roentgenograms and microscopical examination, and in both cases the bones were brittle. No significant difference in the ash and mineral content of the bones could be detected in scorbutic and hypervitaminotic guinea pigs (see table 77). Hyperemia and hemorrhages, particularly muscular hemorrhages in the hind legs, as well as free blood in the knee joints, were present in both cases. Similar findings were revealed in the kidneys by microscopical examination in both cases, such as scattered red blood cells outside the capillaries, free blood in the space of Bowman's capsule and slight degeneration of the renal tubules. Furthermore, the same low content of ascorbic acid in the liver and in the serum was detected in both cases.

It was also shown that similar gross doses of vitamin A proved more toxic to guinea pigs given a scorbutic diet, than to guinea pigs

Table 76.

Ash and Mineral Contents of Femurs in Normal, Hypervitaminotic, and Scorbutic Guinea Pigs.

Conditions of experiment	Guinea pig no.	Sex	Initial body weight, g	Duration of exp., days	Vit. A. dose: I. U. vit. A/day	Vit. A. dose: I. U. vit A/g body wt./day	Ash, % of dry bone	Ca, % of ash	P, % of ash	Fractures
Basal diet (Control)	10	♂	100	30	-	-	53.2	31.1	17.0	0
	17	♂	327	37	-	-	57.5	34.1	18.6	0
	Mean		214	34	-	-	55.4	32.6	17.8	-
Basal diet + excess of vit. A.	9	♂	74	12	10,000	130	45.0	34.1	20.0	0
	11	♂	108	30	15,000	90	53.4	24.9	17.7	0
	12	♂	254	24	10,000	55	52.6	34.6	19.4	0
	13	♂	134	12	50,000	370	39.6	33.8	18.9	+
	14	♂	101	9	17,500	200	48.8	30.7	18.5	0
	15	♂	156	17	17,500	120	51.8	30.3	18.2	0
	18	♂	297	38	50,000	150	60.3	32.6	18.2	0
	19	♂	197	25	50,000	240	52.2	34.5	18.5	+
	24	♂	152	6	60,000	400	47.4	31.9	17.9	0
	26	♀	96	4	35,000	400	36.7	42.0	20.2	0
	27	♂♀	114	8	35,000	300	39.5	29.4	20.0	0
	32	♀	143	8	50,000	350	46.8	35.5	17.3	+
	Mean		152	16	33,250	234	47.8	32.9	18.7	-
Basal diet + excess of vit. A + vit. C (50 mg/day)	25	-	159	7	60,000	400	47.3	29.0	17.7	0
	28	♀	120	11	35,000	340	47.9	25.4	17.2	0
	29	♂♀	105	7	35,000	350	44.4	40.7	17.8	+
	35	♀	134	10	50,000	380	51.2	35.8	18.0	0
	Mean		130	9	45,000	368	47.7	32.7	17.6	-
Scorbutic diet	20	♀	238	38	-	-	49.4	33.3	19.7	0
	21	♀	140	13	-	-	50.1	30.9	17.8	+
	Mean		189	26	-	-	49.8	32.1	18.8	-
Scorbutic diet + excess of vit. A	22	♀	300	16	50,000	235	53.1	30.3	16.5	+
	23	♂	132	13	50,000	400	45.2	33.1	19.5	+
	Mean		216	15	50,000	318	49.2	31.7	18.0	-

given the same usual adequate basal diet, and a combination of the scorbutic diet and excess of vitamin A proved more injurious than scorbutic diet alone.

On the other hand subcutaneous or oral administration of 50 mg ascorbic acid daily offered no protection against the injurious effect of 300—400 I. U. vit. A/g body weight daily in guinea pigs, while the same dose of vitamin C appeared to have some beneficial effect against 115—240 I. U. vit. A/g body weight daily, as judged by clinical examination.

D. Hypervitaminosis A in Rabbits.

The purpose of the following experiments was to examine the effect of excess of vitamin A in rabbits. It has previously been stated by Bomskov (1935) that rabbits are resistent against overdosing with vitamin A.

In the present investigation, one coloured and five white rabbits were used, all of which were taken from the main stock of animals which had lived their entire life in the laboratory on the usual basal diet. During the experiments, the animals were in all cases given the same standard basal diet in unlimited quantities, consisting of hay, bread, water, milk and occasionally greens. The body weights were recorded at intervals during the experiment, and in the case of young growing rabbits the weight gain was also observed about a week prior to the commencement of the experiment. The animals were kept in separate individual cages.

Experiment 49.

In a preliminary experiment, a young white rabbit with an initial body weight of 376 grams was given excess of vitamin A in the form of whale liver oil concentrate (200,000 I. U./g) by dropping pipet during a period of 13 days, another young white rabbit of the same weight being used for control purposes. Except for the vitamin A these two rabbits lived under identical conditions.

The average daily dose of vitamin A was 75,000 I. U. daily, corresponding to approximately 200 I. U. vit. A/g body weight. On this dose an average reduction of the weight of 1.8 g daily was observed, while the control rabbit gained an average of 19 grams daily (see weight graph no. 25).

On the third day of the experiment the rabbit given excess of vitamin A appeared weak and showed lack of activity. From the 4th day it suffered from diarrhea, and the following day alopecia was observed around the mouth. On the 7th day hemorrhages were observed in the skin on the toes at the base of the nails. It hardly moved its legs and the toes appeared stiff and painful. At the same time the previously described symptoms became more pronounced.

From the 15th day of the experiment only ordinary basal diet was given without any excess of vitamin A. The weakness and the unhealthy appearance persisted, however, the weight remained unchanged, and the rabbit died at the end of one month.

Experiment 50.

In another experiment an adult coloured male rabbit was given, in addition to the usual basal diet, vitamin A acetate dissolved in peanut oil (300,000 I. U. vit. A/g) in amounts corresponding to approximately 100,000 I. U. vit. A daily, or approximately 50 I. U. vit. A/g body weight, during a period of 25 days. The oil was given by a specially constructed metal catheter, whereby the entire dose was swallowed without any oil escaping.

Shortly after the commencement of the experiment, a considerable loss of weight was observed. The initial weight of the rabbit was 2150 grams, and at the end of 25 days it was 1750 grams, — the average loss of weight being 16 grams daily (see weight graph no. 26).

From the 10th day of the experiment the rabbit appeared weak, and on the 17th day, marked alopecia was observed around the mouth.

At the end of 25 days the excess of vitamin A was removed from the diet and only basal diet was given during a period of two months, during which time

the symptoms rapidly disappeared. At the end of this period the rabbit appeared healthy and quite normal, and the weight increased from 1750 to 2375 grams (10.4 g daily).

It was then again given excess of vitamin A in the form of whale liver oil concentrate (200,000 I. U./g) in amounts corresponding to an average of approximately 327,000 I. U. vit. A daily (varying between 200,000 and 400,000 I. U.) or approximately 133 I. U. vit. A/g body weight, during a period of 44 days.

During this period the average daily weight increase was 2.4 g. Apart from weakness, observed from the 14th day, no clinical symptoms were detected.

At the conclusion of the experiment blood samples were collected as described on page 22, 24 hours after the last dose of vitamin A, and the following findings were made: Vitamin A: 2.2 I. U./g serum; Ascorbic acid: 0.00 mg/100 ml serum; Hemoglobin: 86 %. In a previous blood sample the serum iron was found to be 156 γ %.

At the end of 44 days the rabbit was killed by intravenous injection of air, and examined immediately after death.

By postmortem examination it was found to be in quite good condition. Apart from marked hyperemia, no significant pathological findings were made. The weight of the left adrenal was 0.011 % of the body weight.

By microscopical examination of the kidneys, adrenals, pancreas, liver and lungs the following findings were made:

Kidney: In some of the glomeruli a few, and in some cases a large number of red blood cells were seen in the space of Bowman's capsule. There was arterial hyperemia, and slight degeneration of some of the renal tubules, the cells of which showed swelling and granularity.

Adrenals: A large number of vacuoles were seen in the cortex, and a few in the medulla.

Pancreas: Large hemorrhages.

Liver: By sudan III staining a large number of smaller and larger sudanophil droplets were seen in and between the liver cells, particularly in the Kupffer cells.

Lungs: No pathological findings.

The liver was extracted by acetone in a Soxhlet's apparatus and the vitamin A content determined spectrographically in the fat. The vitamin A content was 12,423 I. U. per gram liver.

The ash content of the femur was 45.6 % calculated on a dry basis. The Ca and P contents of the ash were 35.2 % and 15.9 % respectively.

Experiment 51.

The purpose of this experiment was to study more closely the lesions produced by excess of vitamin A in young rabbits. For this purpose two young white male rabbits with initial body weights of 700 and 570 g respectively were used, a third female rabbit (720 g) being used for control purposes. The two first mentioned rabbits received in addition to the usual basal diet, excess of vitamin A in the form of whale liver oil concentrate (200,000 I. U./g) as used in previous experiments on rats. The control rabbit was given the same basal diet without any excess of vitamin A. The whale liver oil was given by the previously described metal catheter.

At the beginning of the experiment approximately 200,000 I. U. vitamin A was given daily corresponding to approximately 285 I. U. and 350 I. U. vitamin A per gram body weight.

At the end of 7 days both the rabbits receiving excess of vitamin A had a limping gait, and two days later they appeared unwell.

The smaller of the two rabbits died at the end of 10 days, after a total consumption of 2,000,000 I. U. vit. A corresponding to an average of 200,000 I. U. vit. A daily or 356 I. U. vit. A/g body weight. The average loss of weight was 5 grams daily (see graph no. 27).

By postmortem examination slight alopecia was observed around the mouth. There was marked hyperemia, and small scattered hemorrhages in the intestines. In the lungs there were signs indicating pneumonia. Otherwise no significant pathological findings were made.

By microscopical examination of the kidneys, liver, lungs, spleen, pancreas and intestines the following findings were made:

Kidney: Marked hyperemia. Free blood was seen in the space of Bowman's capsule, which in some places appeared packed with red blood cells. There was slight degeneration of some of the renal tubules, some of which were filled with amorphous masses and a great number of crystals. In some places a few red blood cells were seen outside the capillaries.

Liver: By sudan III staining a great number of large sudanophil droplets were seen in and between the liver cells, particularly in the Kupffer cells. By hematoxylin-eosin staining a large number of vacuoles were seen which seemed to correspond to the sudanophil droplets in the sudan III stained preparation. A number of cells resembling blood macrophages were seen.

Lungs: There was marked thickening of the alveolar walls, hyperemia, exudate in the alveoli containing red blood cells and a large number of granulocytes.

Spleen: The sinuses appeared packed with macrophages containing blood pigment. There was slightly increased amounts of connective tissue in the sinus walls, while the sinuses contained comparatively few red blood cells.

Intestines: No significant pathological findings.

Pancreas: Deposits of blood pigment.

The vitamin A content of the liver was 7,500 I. U. /g. The ash content of the femur calculated on a dry basis was 47.6 %. The Ca and P contents of the ash were 30.4 % and 16.3 % respectively.

The remaining rabbit gradually lost weight, and showed lack of activity. On the 20th day fracture of the right hind leg occurred as a result of a sudden movement of the extremity during weighing (spontaneous fracture) (see ill. 9, 26). Four days later it was practically moribund.

The feces was very dark and the benzidine reaction was positive after 5 seconds. The amount of vitamin A lost through feces was determined in one 24 hour period and found to be 5 % of the gross dose.

A blood sample was collected at the conclusion of the experiment by the technique previously described. The hemoglobin was 84 %, and the vitamin A content was 22 I. U./g serum, as against 82 % and 1 I. U. vit. A/g respectively for the control rabbit.

This rabbit died at the end of 30 days, after a total consumption of 5.2 million I. U. vit. A, corresponding to approximately 173,000 I. U. vit. A daily or 244 I. U. vit. A/g body weight. The average loss of weight was 6.7 g daily (see graph no. 27).

By postmortem examination no subcutaneous or visceral hemorrhages were detected. Apart from marked hyperemia, fiery red lungs and enlarged adrenals, no pathological findings were made by macroscopical examination. The weight of the left adrenal was 0.014 % of the body weight.

By microscopical examination of the internal organs, marked hyperemia was revealed in the kidneys, adrenals, liver, lungs and pancreas. In the kidneys, red blood cells were seen in the space of Bowman's capsule in some places. In the lungs the same findings as described for the previous rabbit were made. By sudan III staining of the liver, massive deposits of sudanophil droplets were seen in and between the liver cells. In the adrenals, dense deposits of sudanophil droplets were seen in the periphery of the cortex (zona glomerulosa and fasciculata), while only moderate deposits were seen in the rest of the cortex.

In the bones similar findings were made as described for the hypervitaminotic rats.

A sample of urine was collected from the urinary bladder by the aid of a thin needle attached to a syringe, in which the Heller's test was distinctly positive, as was the boiling test (Esbach 0.5 ‰), and by microscopical examination of the centrifuged urine, many red blood cells per field of vision were seen.

The liver was extracted by acetone in a Soxhlet's apparatus, and the vitamin A content was found to be 3,156 I. U./g liver, determined spectrographically.

The ash content of the femur was 51.2 % calculated on a dry basis. The Ca and P contents of the ash were 33.9 % and 17.5 % respectively.

By X-ray examination of the long bones in the rabbits receiving excess of vitamin A similar changes were found as described for rats given excess of vitamin A (see ill. 25, 26).

The control rabbit remained healthy throughout the experiment. The average daily weight gain was approximately 13 grams.

By postmortem examination normal organs were found. The weight of the left adrenal was 0.005 % of the body weight.

By microscopical examination of the internal organs and bones, normal conditions were found. By sudan staining of the adrenal a comparatively large number of sudanophil droplets were seen throughout the entire cortex, except for a narrow band just inside the capsule. Sudanophil droplets were also seen in the medulla. By sudan III staining no sudanophil droplets were seen in the liver.

The Heller's reaction in the urine was negative, the vitamin A content of the liver was 537 I. U. per gram, the ash content of the femur was 37.7 % calculated on a dry basis, and the Ca and P contents of the ash were 35.9 % and 18.2 % respectively.

Summary of Results. From experiments 49—51 it may be concluded that excess of vitamin A is also toxic both to young and adult rabbits.

Doses between 50 and 130 I. U. vitamin A/g body weight daily gave rise to toxic symptoms in the adult rabbits. In young rabbits, doses between 200 and 350 I. U. vitamin A/g body weight daily proved lethal in all cases after 10 to 30 days (see table 77).

Clinical symptoms similar to those observed in hypervitaminotic rats, mice and guinea pigs occurred also in rabbits given excess of vitamin A, such as: reduced weight gain — or loss of weight, weakness, scruffiness, alopecia, lack of activity, stiffness and pains in the limbs, limping and fractures. In one case diarrhea and bleeding in the skin was observed.

Postmortem examination revealed hyperemia, enlarged adrenals, visceral hemorrhage, fiery red colour of the lungs, and signs indicating

Table 77.

Clinical Symptoms, and Postmortem Findings in Rabbits given Excess of Vitamin A.

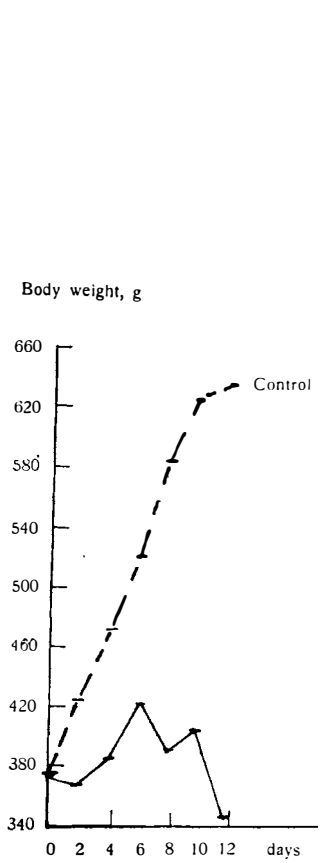
Experiment no.		Daily dose of vit. A		Initial body weight, g	Duration of experiment, days	Weight increase, g/day	Lethal result	Weakness	Lack of activity	Scruffiness	Alopecia	Diarrhea	Hemorrhages in the skin	Stiffness & pains in limbs	Limping	Fracture	Enlarged adrenals	Visceral hemorrhages	Fiery red lungs	Hyperemia	Red blood cells in space of Bowman's capsule	Degeneration of renal tubules	Red blood cells outside capil.	Sudanophil deposits in liver	Hematuria	Proteinuria	Positive benzidine test in feces
		I. U. / day	I. U. / g body weight																								
Exp. 60	(Control)	-	-	-	(15)	+ 19.0	0	+	+	0	+	0	+	0	0	0	-	-	-	-	+	-	-	-	-	-	-
		75,000	200	376	(15)	— 1.8	+	0	0	0	0	0	0	0	0	0	0	0	0	+	-	0	0	0	0	+	+
Exp. 61	a)	100,000	50	2150	(25)	— 16.0	0	+	+	+	+	0	0	0	+	0	-	+	+	+	-	+	+	+	+	-	-
	b)	327,000	133	2375	44	+ 2.4	0	+	0	0	0	0	0	0	0	0	+	0	0	+	+	0	0	+	+	+	+
Exp. 62	(Control)	-	-	720	30	+ 13 0	0	0	0	0	0	0	0	0	+	+	0	+	0	+	+	+	+	+	-	-	-
		173,000	244	700	30	— 6 7	+	+	+	+	+	0	0	0	+	0	+	0	+	+	-	0	0	+	+	+	+
		200,000	350	570	10	— 5.0	+	+	+	+	+	0	0	0	+	0	-	+	0	+	+	0	0	+	-	+	-

Table 78.

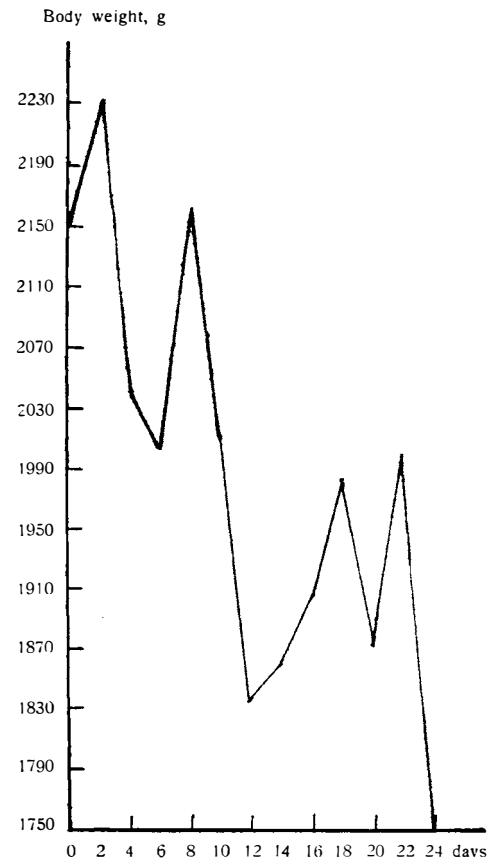
Results of Various Laboratory Examinations in Rabbits given Excess of Vitamin A.

	Conditions of experiments	Daily dose of vit. A		Initial body weight, g	Duration of experiment, days	Vit. A in serum,[1] I. U./g	mg ascorbic acid/100 ml serum	Hb %	Serum iron, γ %	Weight of left adrenal in % of body weight	I. U. vit. A/g liver	Ash, % of dry bone	Ca, % of ash	P, % of ash
		I. U./day	I. U./g body weight											
Exp. 61	Basal diet + excess of vit. A	327,000	133	2375	44	2.2	0.00	86	156	0.011	12423	45.6	35.2	15.9
Exp. 62	Basal diet	-	-	720	30	1.0	0.18	82	-	0.005	537	37.7	35.9	18.2
	Basal diet + excess of vit. A	173,000	244	700	30	22.0	-	84	-	0.014	3156	51.2	33.9	17.5
		200,000	350	570	10	-	-	-	-	-	7500	47.6	30.4	16.3

[1] 24 hours after the last dose of vitamin A.



No. 25.



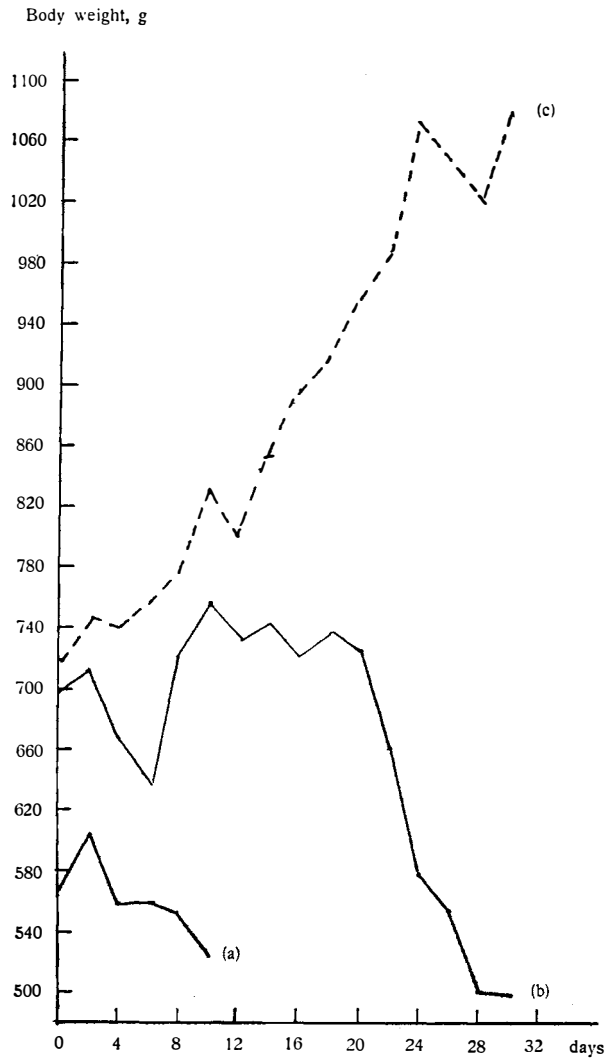
No. 26.

Graph No. 25. Weight graph for young rabbit given 200 I.U. vit. A/g body weight daily, compared with the control.
Graph No. 26. Weight graph of adult rabbit given 50 I.U. vit. A/g body weight daily, during 25 days.

pneumonia. Histologically, hyperemia, red blood cells in the space of Bowman's capsule, slight degeneration of the renal tubules, hemorrhages, vacuoles in the liver, and dense deposits of sudanophil droplets in and between the liver cells were detected.

Hematuria and proteinuria were observed in the one case examined, as well as a positive benzidine reaction in the feces.

The vitamin A level in the blood was increased 24 hours after the last dose of vitamin A. There was no anemia, and the serum iron appeared to be normal. The weight of the left adrenal, calculated in per cent of the body weight, was considerably increased. The vitamin A content of the liver was between 3,000 and 12,400 I. U./g. The ash contents of the femurs were not reduced (see table 78).



Graph No. 27. Weight graphs for young rabbits given 256 I. U. (a) and 244 I. U. (b) vitamin A/g body weight daily, compared with the control (c).

E. Hypervitaminosis A in Birds.

Experiment 53.

In a previous publication (Rodahl (1949)), it was reported that chicken died as a result of eating polar bear liver, and that birds usually avoided eating this liver which was shown to be rich in vitamin A. It was therefore considered desirable to investigate the clinical effect of large doses of vitamin A in birds.

For this experiment 6 young cockerels from the same hatch with initial body weights of approximately 600—900 grams were used, which had been reared on ordinary poultry food. They were divided into two groups, each consisting of three birds, one group being used for control purposes. The two groups lived under identical conditions, and received a basal diet in unlimited quantities, consisting of a

mixture of: oats, maize, dried grass, and a mixture of crushed oats and maize meal boiled in water, with an addition of calcium. In addition to this the experimental birds were given excess of vitamin A orally in the form of whale liver oil concentrate (180,000 I. U. vit. A/g) in amounts corresponding to between 180,000 and 720,000 I. U. vitamin A daily, by a specially constructed metal catheter.

The birds were clinically examined every day, and the weight was recorded every 4th day.

The average daily vitamin A dose throughout the experiment was 208,000 I. U., corresponding to an average of 260 I. U./g body weight (235 to 286 I. U./g body weight). The average daily weight gain was 47.6 g as against 195 g for the controls.

At the end of 5 days it was observed that the combs in two of the experimental birds appeared pale and sore. The following day a trembling was observed in the two mentioned birds, and in one of them the right eye was sore, swollen and practically closed. The mentioned symptoms were observed in all of the experimental birds at the end of 10 days. They looked miserable, scruffy and unkept, the feathers being smeared with feces (see ill. 10 & 11). One of the birds showed signs of vertigo, and walked as if it was intoxicated.

At the end of 15 days all the experimental birds looked particularly miserable. The combs were drooping, and there was marked soreness and swelling around the eyes, which were practically closed, with swelling of the palpebrae and loss of feathers. The tail feathers hung drooping, and they walked with an uncertain swaying movement.

The birds were killed at the end of 18 days and examined immediately after death. Apart from marked hyperemia, and fatty cut surface of the livers, no pathological findings were revealed by macroscopical postmortem examination of the cockerels receiving excess of vitamin A.

By microscopical examination of the kidney, liver, testis, heart and small intestines from one of the control cockerels, no pathological findings were revealed. In all three cockerels given excess of vitamin A, the kidney, liver, pancreas, spleen, testis, heart and intestines were examined histologically and similar findings as described for the hypervitaminotic rats were made:

Kidney: Marked hyperemia, slight degeneration of some of the renal tubules, some of which showed deposits of calcium, and red blood cells were observed in the space of Bowman's capsule.

Liver: Marked hyperemia, and a large number of vacuoles were seen in all cases. When stained by sudan III very dense deposits of large sudanophil droplets were seen throughout the organ, mostly situated between the liver cells, and only very few were detected in the actual liver cells.

In the pancreas, spleen, testis, heart and small intestine, there was marked hyperemia in all cases, but otherwise no pathological findings were revealed.

The average vitamin A content of the livers was 10,800 I. U. (9,300—12,400 I. U.) vit. A/g liver, as against 300 I. U./g liver in the normal control birds.

From this experiment it appears that excess of vitamin A is also toxic to birds, and that 260 I. U. vit. A/g body weight daily reduced the weight gain to approximately ¼ of the normal in young cockerels.

III. Discussion.

From the present investigations it is evident that identical effects were obtained in rats by giving livers rich in vitamin A, liver oils, and highly purified and concentrated vitamin A preparations, when given in equivalent amounts with regard to the vitamin A content. The symptoms increased with increasing doses of vitamin A, and no ill effect whatever was observed in rats given liver oil, where the vitamin A had been destroyed. Finally, none of the fractions of the liver oil, other than the vitamin A, had any ill effect on rats. These findings are in agreement with the results of previous investigations (Rodahl (1949, 1, 2)). Furthermore, excess of pure crystalline vitamin A alcohol (Pavcek, Herbst and Elvehjem (1945)), and crystalline vitamin A acetate (Moore and Wang (1945)) have been found to produce the same toxic effect in rats as natural sources of vitamin A.

The sources of vitamin A used in the present investigations contained practically no vitamin D. In fact, the whale liver oil which was used in the majority of the experiments, had no antirachitic effect whatever. None of the injuries observed could therefore be attributed to excess of vitamin D. In accordance with this, the symptoms produced in these experiments resembled in no way those of hypervitaminosis D.

There appears to be no doubt, therefore that excess of vitamin A is toxic, and that the ill effect observed in rats given excess of the vitamin A concentrates used in the present experiments is due to the vitamin A itself. From the present investigations, it may further be concluded that excess of vitamin A is also toxic to mice, guinea pigs, rabbits and cockerels, while it has previously been stated that rabbits (Bomskov (1935)) are resistent against overdosage with vitamin A.

The same toxic symptoms were observed when excess of vitamin A was administered orally or given by subcutaneous injection. The quantities of natural vitamin A oils required to give rise to toxic symptoms when injected subcutaneously were considerably higher, however, than when given orally. This is probably due to incomplete and slow absorption of the vitamin from the subcutaneous tissue, as the injected oil could be detected encapsuled in the subcutaneous tissue long after the injection. On the other hand, vitamin A emulsions were readily absorbed and gave rise to more pronounced effect than when the natural vitamin A oil was injected. In the first case the effect of equivalent doses of vitamin A was identical when compared with oral administration.

Subcutaneous injection of single massive doses of vitamin A has been found to cause death with cramps in rats by some previous workers (Takahashi et al. (1925), Matsuoka (1934)). In none of our experiments cramps occurred as a result of subcutaneous injection of large quantities of vitamin A oils — up to 1 million I. U. vit. A in a single injection —

when great care was taken not to introduce the oil into the blood stream.

When vitamin A concentrates were applied locally on the skin in mice, toxic symptoms similar to those observed in rats and mice given excess of vitamin A orally, occurred, even when no vitamin A was allowed to be swallowed by the animal. This indicates that under these circumstances, sufficient vitamin A is absorbed through the skin to cause symptoms of hypervitaminosis A in mice.

Identical gross doses of vitamin A oils appeared to prove slightly more injurious to rats when partly mixed in the basal diet, than when given entirely by dropping pipet, or by a specially constructed catheter. In the latter case a larger proportion (approximately 20 % more) of the gross dose of vitamin A was lost through the feces, and it is possible that this may account for the difference in the observed effect. These observations indicate, however, that different experimental procedures may influence the onset and severity of the manifestation of hypervitaminosis A.

Oral administration of single massive doses of vitamin A, apart from causing symptoms of acute intoxication shortly after the administration of the dose, and loss of weight, caused only a slight delayed effect in rats, and produced no pathological changes in the internal organs and bones, although the vitamin A content of the blood reached very high levels. In no case was it possible to produce lethal effect by single doses of vitamin A given during a short space of time — such as 1.6 million I. U. vitamin A given in the course of three hours. Nor did a very high vitamin A level in the blood (31 I. U. vit. A/g serum) in rats in itself prove lethal. No ill effect whatever followed a single subcutaneous injection of 1 million I. U. vit. A in the form of highly concentrated whale liver oil, which was probably due to incomplete absorption of the oil.

It is thus evident that a prolonged administration of excess of vitamin A in "toxic" doses over a period of several days is necessary to produce the changes characteristic for the condition called hypervitaminosis A.

In some cases a distinct improvement of the symptoms occurred in the hypervitaminotic rats in the course of the experiment, in spite of the excess of vitamin A being given continuously in unchanged doses, without it being possible to explain the cause for this.

Toxic effect has not been seen in animals after ingestion of excessive amounts of carotene, probably because it is less well absorbed than vitamin A, and that the conversion of the carotene into vitamin A is not rapid enough to allow sufficient accumulation of vitamin A for the production of toxic symptoms.

With regard to the *toxic dose* of vitamin A, Rodahl and Moore (1943), and Herbst, Pavcek and Elvehjem (1944) found that doses over

15,000 I. U. vit. A daily are toxic to rats. This has been confirmed in the present investigation.

An apparent effect of age has previously been reported by Rodahl and Moore (1943), Moore and Wang (1945) and Walker, Eylenburg and Moore (1947) on the manifestation of hypervitaminosis A in rats given similar gross doses of vitamin A. In the present experiments, the doses of vitamin A were both expressed in terms of I. U. vit. A daily, and I. U. vit. A per gram body weight daily, in young as well as in adult rats. From these experiments it appears that under identical conditions, equivalent daily doses of vitamin A expressed as I. U. vit. A/g body weight had the same effect in rats at all stages of development, although some of the lesions manifested themselves later in adult rats than in young rats.

It is thus obvious that similar daily gross doses of vitamin A would appear to prove less injurious to adult rats than young animals because of the greater body weight of the former. When comparing the effect of excess of vitamin A in animals of different weights, the doses of vitamin A should therefore be expressed as I. U. per gram body weight.

There was also a reasonable agreement in the toxic doses of vitamin A when expressed as I. U. vit. A/g body weight per day in different animals, such as rats, mice, guinea pigs and rabbits. No difference was observed in the effect of excess of vitamin A in the two sexes.

Confirming the statement made by Rodahl and Moore (1943), the clinical picture of hypervitaminosis A was found to vary remarkably with the magnitude and duration of the overdosage. While in all cases doses over 50—100 I. U. vit. A/g body weight daily in addition to the usual adequate basal diet gave appreciable toxic manifestations in rats, doses between 47 and 135 I. U. vit. A/g body weight produced only slight toxic symptoms with moderately reduced weight gain and slight proteinuria and hematuria, and in one case swelling of the palpebrae and soreness around the eyes. Apart from this no other clinical symptoms were observed, even when the mentioned dose was given daily during a period of 56 days. In all cases doses between 200 and 500 I. U. vit. A/g body weight caused pronounced clinical symptoms in rats, and pathological changes, typical for hypervitaminosis A. Doses over 800 I. U. vit. A/g body weight proved lethal at the end of periods varying from 9—30 days in all cases.

These findings are in agreement with the results of previous investigations (Rodahl (1949, 1, 2)).

Similar observations were made in other experimental animals. Thus in mice doses over 60 I. U. vit. A/g body weight proved toxic, and doses between 60—600 lethal. In guinea pigs doses over 50 I. U./g body weight proved toxic and doses between 300—500 lethal in all cases. In rabbits doses between 50—130 I. U./g body weight gave rise to toxic

symptoms, and doses between 200—350 proved lethal. Finally 260 I. U. vit. A/g body weight daily caused toxic symptoms in cockerels at the end of 5—10 days.

The mentioned figures must be taken as maximum values, however, since it was shown in experiments in rats, that approximately 40 % of the ingested amount of vitamin A was lost through feces.

In a previous publication (Rodahl (1949, 2)), where the *symptomatology* of hypervitaminosis A in rats was discussed in detail, the symptoms were divided into symptoms of "acute intoxication" and symptoms of more "chronic intoxication". These observations have been confirmed in the present investigation.

Symptoms of "acute intoxication" may occur within a few hours after single toxic doses in the form of general malady, changes in the pelts, drowsiness, muscular weakness, and decreased activity. Simultaneously, a rapid increase of the vitamin A level in the blood was observed during the first 2—4 hours, followed by a rapid decrease. At the end of 48 hours the vitamin A level of the blood had usually reached normal levels, at the same time as the animals given a single dose, appeared to have recovered. If no further excess of vitamin A was given, it appeared as if the animal recovered without any detectable injurious effect. It is thus probable that the symptoms of "acute intoxication" are related to the sudden large increase of the vitamin A level of the blood.

Continued administration of the mentioned toxic doses during a period of several days, caused symptoms of "chronic intoxication", such as lack of appetite and reduced weight gain or loss of weight, muscular weakness, loss of hair, soreness and bleeding in the skin, swelling of the palpebrae, exopthalmus, stiffness in the limbs, limping, spontaneous fractures and eventually death. Examination of the urine usually revealed proteinuria and hematuria, and in the feces the benzidine test was often positive. In some cases hypochromic or normochromic anemia was observed, while in the majority of cases the hemoglobin values fell within the range of those of the normal control rats (see table 68). The sedimentation rate was reduced (Rodahl (1949, 2)). The fasting blood sugar was reduced in one case and increased in two cases, otherwise the values fell within the range of the normal.

Macroscopical postmortem examination in many cases revealed no significant pathological findings. Otherwise the most constant findings were emaciation, arterial hyperemia, subcutaneous, muscular or visceral hemorrhages, fractures, enlarged adrenals, fatty appearance of the cut surface of the liver, swelling of the visceral lymph glands and widening of the costochondral junction in some cases. In some of the rats the lungs and other internal organs were fiery red. Of the microscopical findings, hyperemia, scattered red blood cells outside the capillaries in the internal organs, slight degeneration of the renal tubules, deposits of sudanophil droplets between the liver cells, particularly in the Kupffer

cells, and slight fatty degeneration of the actual liver cells were the most constant findings. By sudan staining large deposits of sudanophil droplets were usually seen in the adrenal cortex. Microscopical examination of the long bones revealed hyperemia, subperiostal hemorrhages, and great irregularity of the bone structure with destruction of bone spicules and signs of increased osteoclastic activity. In the teeth marked hyperemia and in some cases hemorrhages were seen in the pulp, as well as vacuoles and deposits of calcium. The odontoblasts were irregularly arranged and appeared degeneratedt in some cases. In some cases there was degeneration of the pulp cells, and amorphous calcification of the inner part of the dentine.

Systematical X-ray examination of the long bones at various stages of the experiment, showed characteristic changes, which will be discussed later (see page 188).

The toxic symptoms caused by excess of vitamin A were essentially similar in the different types of experimental animals examined.

It is a question whether all these symptoms may be considered as directly caused by excess of vitamin A as such (specific symptoms), or whether some of them may be indirect rather than direct results.

Absence of the normal weight gain is one of the first and most constant symptoms in hypervitaminotic rats. This was found to be associated with reduced food intake as a result of lack of appetite. This lack of appetite is not caused by the oil-food mixture being less palatable to the rats, since the same oil, when the vitamin A had been destroyed, had no adverse effect on the appetite or the weight gain. The lack of appetite observed in hypervitaminoss A must therefore be considered as being attributed to the action of excess of vitamin A.

It would seem reasonable to assume that the reduced food consumption in the nost pronounced cases might influence the general condition of the animals and partly be responsible for some of the general symptoms such as scruffiness, reduced resistance against infection etc. On the other hand a number of the general symptoms are observed in direct connection with the first massive doses of vitamin A, before the effect of inanition would be noticeable. Finally, when massive doses of vitamin A are given, the animals often die suddenly within a short period of time, in spite of food being taken, during which period the effect of inanition would hardly have been significant. It would therefore seem likely that the reduced food consumption plays no significant part in the production of the symptoms of hypervitaminosis A. The importance of this factor could easily be studied further by limiting the food intake of the control rats to that of the test animals. This will be subject to a later investigation.

Symptoms similar to the muscular weakness, and lack of activity observed in hypervitaminotic rats, have also been described in man following ingestion of large amounts of vitamin A (Rodahl (1949, 2)), in

which case violent headache is a very pronounced symptom. In this connection it is of interest to note that marked hyperemia in the meninges is often seen in hypervitaminotic animals, which may possibly be the cause of the mentioned headache in man.

Alopecia is a very frequent symptom in hypervitaminotic animals. Rats receiving large doses of vitamin A oils orally suffered from loss of hair around the mouth and nose, and also elsewhere on the body. In some cases large areas of the body were affected. General alopecia also occurred in rats injected with vitamin A subcutaneously, while alopecia did not occur in healthy rats when the vitamin A oil was applied locally on the skin between the shoulder blades, in such a way that the rats were unable to reach the spot where the oil was applied. Alopecia was observed even when only a few drops of highly concentrated sources of vitamin A were given orally, and when the oil was given with the aid of a specially constructed catheter, so that no oil escaped, while no loss of hair was observed in rats given the oil where the vitamin A had been destroyed. Alopecia may therefore be taken as a specific symptom of hypervitaminosis A.

Histological examination of pieces of the skin taken from areas with marked alopecia, revealed in a number of cases marked hyperemia and massive hemorrhages around the root sheaths of the hair (see ill. 38). This may probably be considered as the cause of the alopecia.

Soreness in the skin often with blood crusts and small hemorrhages, particularly around the nose and eyes, occurred frequently in animals given excess of vitamin A orally, and also in rats given excess of vitamin A by subcutaneous injection, while it was never observed in animals given the vitamin A oil freed of its vitamin A content. This symptom also persisted in some cases in hypervitaminotic rats for a considerable time (50 days) after the excess of vitamin A was removed from the diet (Rodahl (1949, 2)). It is not likely, therefore, that this symptom could be caused by any mechanical or local effect of the oil, but it seems reasonable to consider this as a specific symptom of hypervitaminosis A as well. Histological examination in several cases revealed inflammatory processes in the skin with leucocytic infiltration. In this connection it may be of interest to note that in mice, where vitamin A concentrates were applied locally on the skin, large encapsuled abscesses containing necrotic tissue and neutrophil granulocytes developed subcutaneously underneath the area where the vitamin A had been smeared.

Several observations seemed to indicate a lowered resistance against infection in hypervitaminotic animals. Thus pneumonia occurred in several cases both in hypervitaminotic rats, guinea pigs and rabbits, while none of the control animals suffered from pneumonia. In this connection it may be mentioned that sudanophil deposits in some cases were detected in the lung tissue of hypervitaminotic rats (ill. 37). In some cases abscesses with pus secretion developed at the places of injection in rats

given excess of vitamin A subcutaneously, while no such abscesses were observed in any of the rats injected with peanut oil.

It would seem possible that the pneumonia in animals given excess of vitamin A by pipet or by catheter, might be due to the mechanical effect of the vitamin A oil, which possibly might have been forced into the respiratory tract during feeding. On the other hand, none of the rats given the oil where the vitamin A had been destroyed, by the same method of feeding, developed pneumonia. In the latter case no sudanophil deposits were detected in the lungs.

Of the eye symptoms, both the swelling of the palpebrae and exophtalmus often occurred at irregular intervals, and denudation of the palpebrae and of the skin around the eyes was frequently observed. While the swelling of the palpebrae appeared to be caused by local oedema, the reason for the exophtalmus could not be detected in the present experiments. Similar to the alopecia and the soreness of the skin, the swelling of the palpebrae and the exophtalmus also appeared to be the result of the action of excess of vitamin A, since these lesions were also observed in rats given excess of vitamin A by subcutaneous injection, — while these symptoms were absent in rats given the oil freed of its vitamin A content orally.

Signs of pathological changes in the extremities in the form of stiffness in the limbs, limping or fracture were present in all types of experimental animals examined in the present investigations. In most cases stiffness, signs of pains in the limbs, and limping were caused either by fracture or hemorrhages (muscular or periostal) or both. In some cases, however, distinct symptoms indicating pains in the legs (stiffness, limping, crying by touching or manipulation) were present without fractures being detected by X-ray examination or bleeding being observed by macroscopical postmortem examination. In these cases, small subperiostal hemorrhages, or hemorrhages in the bones themselves, or other pathological changes in the bones which were not detected by macroscopical postmortem examination, might possibly be the cause of the pains.

Contrary to some previous publications (Moore and Wang (1945), and others) no significant difference in the incidence of spontaneous fractures in young and adult animals was detected in the present experiment, although the fractures developed later in adult than in young rats. Since the occurrence of spontaneous fractures in hypervitaminotic animals, to some extent, depends on the activity of the animal, — and thereby the exposure to such traumata necessary to produce the fractures, the pathological changes in the bones causing the fractures should be emphasised rather than the fractures themselves, — which represent the final stage in the pathological process leading to the tendency to spontaneous fractures.

While Strauss (1934) reported that the bones in hypervitaminosis A showed retardation of osteogenesis, cessation of endochondral bone formation, — but no general resorbtion of bone, — and that osteoclasis was only marked in the regions of fractures, Wolbach and Bessey (1942) describe the histological findings as decalcification and osteoporosis, accompanied by large numbers of osteoclasts, and report the osteoporosis to be most marked in the regions where the remodelling of bone is a normal growth process, — i. e. the ends of the long bones.

In agreement with previous findings (Rodahl (1949, 2)) systematical X-ray examinations of the long bones in hypervitaminotic rats at various stages of the experiments showed that the bones gradually became abnormally thin, while the length of the bones appeared to remain more or less unaffected. This reduction in the diameter of the bones, which occurred early in hypervitaminosis A, particularly involved the bone shaft, while the epiphyses appeared to be more or less of normal width. This was particularly noticeable at the proximal end of the tibia and the fibula, where the epiphyses looked as if they were swollen compared with the abnormally thin shaft. The cortical shadow was also abnormally thin, and was usually only represented by a narrow white line. Very often it was observed that the cortical shadow was absent at both ends of the bones, and when the periost was removed at postmortem, only spongy and no compact bone was found at the mentioned places. Simultaneously it was observed that the epiphyseal line became abnormally narrow, as compared with normal rats of similar age, and in some cases it was practically closed. As judged by the roentgenograms, the bones generally appeared normally calcified ,but lines of denser calcification were seen in the epiphyses as well as at the epiphyseal border of the metaphyses in all the long bones. Histological examination of the bones revealed findings corresponding to the changes observed in the roentgenograms, and the pathological changes in general were most pronounced towards the epiphyses.

In very young rats, roentgenograms also showed a broad, dense shadow around the centre of ossification, an irregularly broadened, intensely calcified zone at the epiphyseal ends of the tibia, and glass transparency of the shaft.

Both in young and adult rats, the diameter of the femur was generally less in hypervitaminotic animals than in normal control rats, when bones of the same length were compared in the two cases, and the diameter measured at corresponding places at postmortem (see ill. 12).

The histological picture of the bones was characterised by marked hyperemia, periostal or subperiostal hemorrhages, and in some cases hemorrhages in the actual bone, as well as irregularity of bone structure with signs of destruction of bone spicules, increased number of osteoclasts, and thinning of the compact bone. Although the bone often appeared irregularly calcified, as judged by histological examination,

there appeared to be no isolated removal of calcium. In agreement with this, chemical analysis of the femurs usually showed no reduction in the calcium content of the ash, while the ash content of the dry bone in some cases was reduced, and generally showed considerable individual variations. This may not be surprising in view of the frequently occurring fractures and hemorrhages in the bones.

In accordance with a previous statement (Rodahl (1949, 2)), it thus appears that there was no significant decrease in the mineral content of the bones in hypervitaminotic animals, as compared with the normal control, at the same time as fragility and a tendency to spontaneous fractures occurred. This fragility, therefore, is hardly caused by "decalcification" of the bones, as is stated in earlier publications on hypervitaminosis A. Nor was it found that excess of vitamin A caused any softening of the bones, as described by other workers. On the contrary it was found that the bones proved to be abnormally brittle, so that they broke easily by grasping with an ordinary forceps at postmortem examination. The teeth were also brittle.

As judged by roentgenograms and microscopical examination, it appeared as if destruction and absorption of the bone substance occurred, while the remaining bone, although irregular in structure, appeared to contain normal richness of calcium and normal density of bone cells. This may well explain the fact that the calcium and phosphorus contents of the ash in the remaining bone appeared unaltered in hypervitaminotic animals.

It would seem natural to consider these bone abnormalities in relation to the observed increased number of osteoclasts, particularly in view of the observation made by Barnicot (1948) who found that local application of crystalline vitamin A acetate caused intense osteoclastic bone absorption, and who suggests that the fractures observed in rats given massive doses of vitamin A may be attributed to local action on the bones. So far the local action on the bones of vitamin A alcohol has not been investigated. Furthermore only small amounts of vitamin A were detected in the bone marrow and in extracts from bones in hypervitaminotic rats in two cases examined. This problem, therefore, should be subject to further investigations.

Wolbach (1946) considers that the bone remodelling is greatly accelerated in hypervitaminosis A, and that a defective tissue is laid down, which fractures easily. On the other hand, it seems probable that at least some of the observed bone changes may be explained as the result of the hemorrhages, particularly those which occur around the periostal blood vessels, and lack of nutrition caused by insufficient blood supply. Furthermore, according to Boyd (1940), hyperemia may lead to rarefaction of bone. Marked hyperemia was almost constantly observed in the bones in hypervitaminotic animals, and this factor, therefore, cannot be overlooked as a possible cause for some of the pathological changes

in hypervitaminosis A. In fact a combination of several factors may be the most likely explanation, since other abnormalities as well, such as hemorrhages in the bones, may also cause absorption and remodelling of bone (Harris (1933)), as already mentioned.

In most cases the fractures occurred near the end of the long bones. The line of fracture was either oblique or transverse. In some cases the epiphyses were detached. As judged by roentgenograms and histological examination, the fractures in most cases occurred at the places where the compact bone was thinnest, and where it would be expected to offer less resistance against breakage. In some cases, however, fractures occurred at places where the compact bone was thicker, thus indicating an abnormal fragility of the bones (ill. 50). In this connection it is of interest to note that the bones generally appeared abnormally brittle as already described (see page 189).

Healing of the fractures usually took place in all cases where the animal survived, just as quickly as healing of artificial fractures produced in healthy rats for comparison, in spite of the vitamin A being given continuously in unchanged doses. In many cases abnormally rich callus formation was observed (ill. 15, 16 & 18).

Certain features of the bone abnormalities in hypervitaminosis A may superficially resemble the condition of ostitis fibrosa generalisata, — such as the bone absorption, spontaneous fractures and ready healing of fractures. In hypervitaminosis A, however, no abnormalities in the calcium and phosphorus contents of the blood have been detected and there is no softening of the bones, no cyst formation and there is no fibrosis of the marrow.

The tendency to bleeding (hemorrhagic diathesis) is a prominent feature in the clinical and pathological picture of hypervitaminosis A. In the present experiments there appeared to be no significant difference in the incidence of hemorrhages in young and adult animals when given equivalent doses of vitamin A. The hemorrhages appeared to be quite general, both subcutaneous, muscular and visceral and varied from small petechia in the skin, — or scattered red blood cells outside the capillaries in the internal organs, to massive blood extravasations and the formation of large hematomas. Most of these hemorrhages, except around the fractures, were scattered and relatively small, however, and they only appeared fatal in very few cases. There appeared to be general tendency to bleeding, — both in the parenchymatous organs, in the bones, mucous membranes, and into the serous cavities, such as the pericardium and the pleura, through the kidneys and intestines, as well as through the roughness of the skin around the mouth and eyes. Epistaxis and bleeding from the gums were also observed.

Hemorrhages often occurred in places where no serious injury was likely to start the bleeding, such as in the internal organs like the liver,

adrenals, lungs, and in the pericardium and the pleura, and additional supply of vitamin K (Rodahl (1949, 2)) had no beneficial effect on the hemorrhages.

Histologically, scattered red blood cells were seen outside the capillaries in the internal organs such as the liver, kidney and lungs, and in some cases also the adrenals, as well as in the bones, indicating an abnormal permeability of the capillary wall for the red blood cells. By the usual staining methods, no morphological abnormalities were detected in the vessel walls, however. Deposits of blood pigment were also detected in the mentioned organs, as well as in the pancreas and spleen in some cases.

In the kidneys, erythrocytes were frequently observed in the tubules and in the space of Bowman's capsule, indicating an abnormal permeability of the capsule for red blood cells. This may well explain the hematuria, which was a frequent finding in hypervitaminotic rats. On the other hand, the observed deposits of calcium in the degenerated tubules may possibly cause focal lesions of the tubular wall, resulting in hemorrhages into the tubular lumen. Both these factors might possibly be taken as the cause of the hematuria, which sometimes was very marked.

The benzidine test in the feces was positive in the hypervitaminotic rats, in the majority of cases examined, while it was usually negative in the normal control animals. Slight hemorrhages in the intestinal wall, and scattered red blood cells outside the capillaries were observed in the mucous membrane of the intestines in some cases, but no ulcerations were observed in the intestinal tract. It is a question whether the small scattered hemorrhages observed would be sufficient to explain the positive benzidine test in the feces. On the other hand, bleeding in the mouth or nose was observed in some cases, which might give rise to positive benzidine test in the feces.

Examination of the blood showed a considerable individual variation in the hemoglobin values in rats given massive doses of vitamin A (see table 68). In some cases distinct hypochromic or normochromic anemia was observed in young and adult hypervitaminotic rats. In the majority of cases, however, the hemoglobin values fell within the range of the normal control rats, and there appeared to be no direct relation between the magnitude of the overdosage with vitamin A and the reduction in the hemoglobin values. A considerable reduction of the serum iron was only detected in one case. In some cases reduced hemoglobin values were associated with considerable hemorrhages, which might possibly explain the anemia as a hemorrhagic anemia. This was not always the case, however, since in some instances anemia was observed without any macroscopical hemorrhages being detected. According to Nordenson (1944), the hemorrhagic anemia is usually normochromic,

but in the course of remission the colour index may be reduced until complete restitution has occurred.

In the present investigation the hematopoietic system was not studied. In view of the above mentioned preliminary findings, however, the blood and the hematopoiesis in hypervitaminotic animals should be made subject to detailed examinations.

No significant difference was detected in the differential blood counts in hypervitaminotic rats, compared with normal rats. Nor were any abnormalities detected in the contents of calcium, phosphorus, total base, potassium or urea of the blood in hypervitaminosis A.

It has previously been reported (Bomskov and Sievers (1933), Rodahl (1949, 2)) that the sedimentation rate is reduced in hypervitaminosis A. The cause of this is unknown.

In one case the fasting blood sugar was reduced, and in two cases it was considerably increased in hypervitaminotic rats. In this connection it may be of interest to note, that marked hyperemia was not only observed in the pancreas tissue, but also in the actual islands of Langerhans, and in some cases signs of old hemorrhages were detected in the pancreas. Hypertrophy of the island tissue of the pancreas in hypervitaminosis A has been reported by Cornil, Chevallier and Paillas (1939). In view of this it seems desirable to devote further interest to the pancreas in hypervitaminosis A.

Proteinuria was a very frequent finding in hypervitaminotic rats, although it was not usually very marked. As a rule the Esbach test did not show more than about 0.5—1 $^{0}/_{00}$ protein after 24 hours. At the same time slight degeneration of the convoluted and collecting tubules of the kidney was a rather constant finding in rats given excess of vitamin A. There was also swelling of the palpebrae (oedema) and the excreted amount of urine in 24 hours was generally less in hypervitaminotic rats, as compared with the control animals. As previously pointed out (Rodahl (1949, 2)) it would seem natural to consider the described histological changes in the kidneys in relation to the observed proteinuria, and the observed indications of disturbances in the renal function and fluid balance.

It might be questioned whether the proteinuria and the slight degenerative changes in the renal tubules may be taken as a direct result of excess of vitamin A. This appears to be the case, however, since the renal damage was also observed in rats receiving moderate excess of vitamin A, and which, apart from very slightly reduced weight gain, appeared to be in general good condition.

Hypervitaminotic rats were considerably less fertile than the normal control rats, or rats given excess of vitamin A in "subtoxic" doses. This appears to be due to the hypervitaminotic condition of the mother, since no improvement of fertility occurred when hypervitaminotic females were

mated with normal males. Furthermore the spermatogenesis appeared to be unaffected and no significant pathological conditions were detected in the genital organs of the male rats, except for priapismus in animals with pronounced symptoms.

No abnormalities were detected in the ovaries of hypervitaminotic rats, which could be taken as the cause of the sterility. No abortion or abnormal uterine hemorrhages were observed in the present experiments, while previous workers (Hartwell (1927); Moore & Wang (1945)) have reported a high incidence of uterine hemorrhage in hypervitaminotic rats. On the other hand there appeared to be absence of libido in rats given massive doses of vitamin A. In this connection it may be of interest to note that Sherwood and collaborators (1936) reported that excess of carotene stops oestrus and libido in rats.

In the cases where young were born by hypervitaminotic mothers, the young were of normal size and weight, and apart from weakness and dryness of the skin in some cases, appeared quite normal. Examination of the vitamin A content of unborn and newly-born young from hypervitaminotic mothers showed that a considerable transplacental transfer of vitamin A must have taken place, since the vitamin A content of the liver, — and of the whole foetus, was 4—5 times higher in these cases than in normal control foetus. This transfer of vitamin A seems, however, to be insufficient to cause hypervitaminosis A in the foetus during intra-uterine life.

There is a very high mortality of the young from hypervitaminotic mothers during the lactation period, and symptoms of hypervitaminosis A were observed in the young a few days after birth. This seems to indicate that the vitamin A transferred to the young through the mothers milk, which was found to contain huge amounts of vitamin A, is sufficient to cause hypervitaminosis A in the young, since the symptoms and postmortem findings observed in the young during the lactation period were identical with hypervitaminosis A, and no other findings were detected which could be taken as the cause of death. A rapid improvement took place in the young when the mother's milk was exchanged by ordinary basal diet in the one case examined.

With regard to the relation between hypervitaminosis A and the action of other vitamins, it may be concluded from the present experiments that additional supply of vitamin B_1 had no influence on any of the symptoms of hypervitaminosis A. From these experiments it is further evident that the condition of hypervitaminosis A did not affect the development of rickets in rats kept on a rachitic diet, nor did it hinder the protective effect of vitamin D given prophylactic to rats kept on a rachitic diet. Finally, excess of vitamin A did not prevent the ricket-curing effect of vitamin D in rachitic animals, as judged by the roentgenograms, although the ash content of the bones in these cases did not

rise to normal values. On the other hand excess of vitamin A proved more injurious to rats given a rachitic diet, than to rats given the usual adequate basal diet.

Light, Alscher and Frey (1944) found that hypervitaminosis A in rats was associated with a pronounced hypoprothrombinemia which could be corrected by giving vitamin K. This has been confirmed by Walker, Eylenburg and Moore (1947). In a previous investigation (Rodahl (1949, 2)) additional supply of vitamin K was found to have no influence, however, on the incidence of hemorrhages or any of the other symptoms of hypervitaminosis A.

In the present investigation the prothrombin time was prolonged in a number of cases in hypervitaminotic rats, although it was not increased in any of the examined cases of hypervitaminotic rats given additional supply of vitamin C. No direct relationship could be detected between the magnitude of the overdosage with vitamin A and the prothrombin time. In some cases, prolonged prothrombin time was associated with anemia, but in no case with macroscopical hemorrhages. On the other hand, hemorrhages were detected in two cases when the prothrombin time was normal. It is thus evident that the hemorrhages in these cases cannot be explained by hypoprothrombinemia. On the other hand, in the majority of cases, increased prothrombin time was associated with increased serum colour, as determined by Meulengracht's method. This observation may possibly indicate that the observed prolonged clotting time might depend on some form of hepatic damage or abnormalities caused by excess of vitamin A, affecting the prothrombin production, or the formation and excretion of bile acids, thus affecting the absorption of vitamin K from the intestines. In this connection there is reason to emphasise the histological abnormalities detected in the liver, the dense deposits of sudanophil droplets between the liver cells, — particularly situated in the Kupffer cells of the reticulo-endothelial system, and the fatty degeneration of the actual liver cells to some extent.

It must be borne in mind, however, that the Meulengracht's test is not specific, and that substances other than bilirubin may cause an increase in the yellow colour.

It seems evident that it is necessary to look for other factors than vitamin K deficiency in order to explain the tendency to bleeding in hypervitaminosis A.

The similarity between the clinical picture of hypervitaminosis A in rats, and that of scurvy has been pointed out by Vedder and Rosenberg (1938), who found that ascorbic acid offered protection against excess of vitamin A, — and by Moore and Wang (1945) who failed, however, to detect any abnormality in the ascorbic acid metabolism in their hypervitaminotic rats, or to produce any beneficial effect with 50 mg ascorbic acid daily in rats receiving excess of vitamin A.

As is evident from the present investigations, the clinical and postmortem findings in our hypervitaminotic animals have produced further evidence of the similarity between hypervitaminosis A and scurvy. A number of these clinical symptoms as well as postmortem findings were identical wth those reported to be observed in human scurvy, such as: muscular weakness, pains in limbs, scattered small hemorrhages apparently due to abnormal capillary permeability, hematuria, intestinal bleeding with diarrhea, subperiostal hematomata, epistaxis, blood extravasations into the muscles, susceptibility to infection, inconstant hypochromic anemia, hepatic and renal damage, widening of the costochondral junction, certain X-ray findings in the long bones, and changes in the teeth such as hyperemia, hemorrhages, vacuoles and deposits of calcium in the pulp, amorphous calcification of the inner part of the dentine, as well as degeneration and disarrangement of the odontoblasts. Even the microscopical picture of the long bones may to some extent resemble that of scurvy. In the majority of cases, abnormally low ascorbic acid contents were found in the serum, adrenals and liver (see tables 48 and 49).

The clinical picture and the postmortem findings in guinea pigs given excess of vitamin A, and in those given a scorbutic diet, were very similar. Thus in both cases there was absence of normal weight gain, hyperemia and hemorrhages, — particularly muscular hemorrhages in the hind legs, as well as free blood in the knee joints. The bone abnormalities were similar, as judged by roentgenograms and microscopical examination, and in both cases the bones were brittle. The ash and mineral contents of the bones were practically identical. Similar findings were revealed in the kidneys by microscopical examination, such as scattered red blood cells outside the capillaries, erythrocytes in the space of Bowman's capsule, and slight degeneration of the renal tubules. Finally, the same low content of ascorbic acid in the liver and in the serum was detected in both cases. Furthermore, similar gross doses of vitamin A proved more toxic to guinea pigs given a scorbutic diet than in those given the usual adequate basal diet.

It is thus evident that prolonged administration of excess of vitamin A in experimental animals produces a condition which largely resembles scurvy, although abnormality in the vitamin C metabolism cannot in any way be considered as the sole causitive factor in hypervitaminosis A. Furthermore, large doses of vitamin C did not offer any significant protection against the injurious effect of massive doses of vitamin A, although additional supply of vitamin C had a beneficial effect against moderate excess of vitamin A.

In this connection it is worth mentioning, however, that according to Szent-Györgyi and Scarborough (quoted by Bicknell and Prescott (1947)), scurvy may be considered as a combined deficiency of vitamins C

and P. It would therefore be of considerable interest also to examine whether additional supply of vitamin P has any effect on the condition of hypervitaminosis A, particularly on the hemorrhages.

From the present investigation it appears that, although the occurrence of the symptoms of "acute intoxication" coincided with the rapid rise of the vitamin A level of the blood following the first dosage of vitamin A, no direct relation between the vitamin A level in the blood or the vitamin A content of the liver and the manifestation or severity of clinical symptoms of hypervitaminosis A could be detected. Thus it appears possible by giving moderate excess of vitamin A in subtoxic doses, to bring the vitamin A content of the liver up to the same magnitude as in animals given distinctly toxic doses, without any significant injurious effect. On the other hand it appears as if the most significant pathological changes are detected in the organs of hypervitaminotic animals, where the storage of vitamin A is highest, particularly in the liver and kidneys. This may indicate a possible direct local injurious effect of excess of vitamin A which appears even more likely on the basis of the demonstration of the local effect of vitamin A on the bones reported by Barnicot (1948). If this is the case, the observed pathological changes in the internal organs may in their turn give rise to secondary symptoms. The possible relation between the marked sudanophil deposits in the liver and the formation of prothrombin and bile acids, and between the degenerative changes in the kidney and the proteinuria and signs of disturbances in the renal function, have already been mentioned.

If the scurvy-like symptoms in hypervitaminosis A were to be taken as the result of vitamin C deficiency, the cause of this deficiency still remains to be explained.

Theoretically, there are several ways in which a deficiency of vitamin C might develop: a reduction of the synthesis or intake of vitamin C, an increased vitamin C requirement, abnormal destruction of vitamin C in the organism, or abnormally increased excretion through the urine, or finally absence of the ability of the cells to utilise the ascorbic acid.

If it is assumed that in rats which normally synthesise sufficient vitamin C to satisfy the normal requirement, the ascorbic acid is synthesised in organs such as the adrenals, it would seem natural to consider the observed pathological changes in the adrenal cortex in relation to the reduced ascorbic acid content of the blood and internal organs in hypervitaminotic rats, as a possible indication of reduced ascorbic acid synthesis. The results of the present investigations, however, allow no definite conclusion to be made on this point.

Mapson and Walker (1948) have stated that the reduced urinary excretion and tissue concentration of ascorbic acid found in vitamin A deficient male rats can be attributed to the reduction of food intake which

accompanies this condition, although restriction of food intake did not reduce the blood values for ascorbic acid of non-deprived male albino rats to the level found in similar vitamin A deficient animals. It is therefore a question whether the reduced food consumption might be responsible for the reduced vitamin C content of the blood and internal organs in hypervitaminotic rats. This should be subject to further investigations.

The condition of hypervitaminosis A presents a highly complex clinical picture. Several of the clinical symptoms have been found to correspond to histological changes attributed to excess of vitamin A. Nevertheless the mechanism of the toxicity on the whole is still quite obscure.

On the basis of the present findings, certain factors may be taken into consideration, however. Certain evidence seems to suggest abnormalities in some of the internal secretory organs, such as the pancreas and the adrenals, which should be subject to further investigations. The cause of the hyperemia, which was a prominent finding in hypervitaminosis A, and the significance of this hyperemia for the manifestation of the different lesions, should be further investigated. Finally, the evidence of direct local action of excess of vitamin A in the internal organs and the osseous system should be examined further.

In view of the moderate histological changes observed in the internal organs, it seems likely to consider the mechanism of the toxic effect as some form of functional disturbance, since in most cases none of the pathologico-anatomical findings detected in the hypervitaminotic animals, considered separately, could be taken as the cause for death.

IV. Summary and Conclusion.

In the present investigation the effect of excess of vitamin A has been studied in 216 rats, 30 guinea pigs, 18 mice, 4 rabbits, and 3 cockerels.

The injurious effect in rats of excess of the vitamin A concentrates used in the present experiments was found to be attributed to the action of vitamin A.

Excess of vitamin A was also toxic to mice, guinea pigs, rabbits and cockerels.

Different Experimental Procedures.

Oral administration of excess of vitamin A produced the same toxic symptoms as subcutaneous injection, although the quantities of natural vitamin A oils required to cause toxic symptoms, were considerably higher when given subcutaneously. This was probably due to incomplete

and slow absorption of the vitamin from the subcutaneous tissue. When vitamin A emulsions were injected subcutaneously, however, the effect was identical with that of oral administration of equivalent doses of vitamin A concentrates.

Contrary to the findings made by Takahashi et al. (1925) and Matsuoka (1934), cramps did not occur as a result of subcutaneous injection of large quantities of vitamin A oils.

Local application of vitamin A concentrates on the skin in mice gave rise to toxic symptoms similar to those observed in experimental animals given excess of vitamin A orally. (Experiment 38.)

Identical gross doses of vitamin A oils appeared to prove slightly more injurious to rats when partly mixed in the basal diet, than when given entirely by dropping pipet or by catheter. In the latter case a larger proportion of the given dose of vitamin A was lost through the feces. (Experiment 8.)

Oral administration of single massive doses of vitamin A in rats only caused symptoms of acute intoxication shortly after the administration of the dose, and only a slight delayed effect with no detectable pathological changes in the internal organs and bones. Single massive doses of vitamin A, — such as 1.6 mill. I. U. vit. A given in the course of three hours, had no lethal effect. (Experiments 13, 14.)

No ill effect whatever followed a single subcutaneous injection of 1 mill. I. U. vit. A, which was probably due to incomplete absorption of the oil. (Experiment 11.)

Prolonged administration of excess of vitamin A in toxic doses over a period of several days was necessary to produce the changes characteristic for the condition called hypervitaminosis A.

No significant effect of age or sex was observed on the clinical picture of hypervitaminosis A in rats given equivalent daily doses of vitamin A expressed as I. U. vit. A/g body weight, although some of the lesions manifested themselves later in adult rats than in young rats.

Toxic Doses.

In rats gross doses over 50—100 I. U. vit. A/g body weight daily gave appreciable toxic manifestations. While doses between 47 and 135 I. U. vit. A/g body weight produced only slight toxic symptoms with moderately reduced weight gain, and slight proteinuria and hematuria, doses between 200 and 500 I. U. caused pronounced clinical symptoms. Doses over 800 I. U. vit. A/g body weight proved lethal in all cases at the end of periods varying from 9 to 30 days. Similar observations were made in other experimental animals, and there was a reasonable agreement in the toxic doses of vitamin A when expressed as I. U. vitamin A/g body weight per day in different animals, such as rats, mice, guinea pigs and rabbits.

Symptomatology.

In agreement with previous findings (Rodahl (1949, 2)), the symptoms following ingestion of toxic doses of vitamin A in rats may be divided into two categories:

a) Those of "acute intoxication", which occurred in direct connection with the first dose, such as: general malady, changes in the pelts, drowsiness, muscular weakness, and reduced activity. These symptoms are probably related to the sudden large increase of the vitamin A level in the blood.

b) Those of "chronic intoxication" following continued administration of the mentioned toxic dose during a period of several days, such as: lack of appetite and reduced weight gain or loss of weight, muscular weakness, loss of hair (ill. 2, 3), soreness and bleeding in the skin, swelling of the palpebrae, exophtalmus (ill. 4), stiffness in the limbs, limping, spontaneous fractures (ill. 1), and eventually death.

Examination of the urine from hypervitaminotic rats, usually revealed proteinuria and hematuria. In the feces the benzidine test was usually positive.

Examination of the blood revealed in some cases hypochromic or normochromic anemia, while in the majority of cases the hemoglobin values fell within the range of those of the normal control rats (table 68). A considerable reduction of the serum iron was only detected in one case. No significant difference was detected in the differential blood counts in rats given excess of vitamin A in addition to the adequate basal diet, compared with normal rats (table 69). No abnormalities were detected in the contents of calcium, phosphorus, total base, potassium or urea of the blood (see page 135). The fasting blood sugar was reduced in one case and increased in two cases, otherwise the values fell within the range of the normal. In the majority of cases the ascorbic acid content of the serum was reduced (table 49).

Systematical X-ray examination of the long bones at various stages of the experiment, showed gradual thinning of the shaft, while the length of the bones remained more or less unaffected. The bones appeared normally calcified, but lines of denser calcification were seen in the epiphyses as well as at the epiphyseal border of the metaphyses. The cortical shadow was abnormally thin, particularly towards the metaphyses, where it was often absent (ill. 13 c, 14). In very young rats, roentgenograms also showed a broad dense shadow around the centre of ossification, and irregularly broadened intensely calcified zones at the epiphyseal ends of the tibia, and a glass transparency of the shaft (ill. 19).

Macroscopical postmortem examination in many cases revealed no significant pathological findings. Otherwise the most constant findings

were emaciation, arterial hyperemia, subcutaneous, muscular or visceral hemorrhages, fractures, enlarged adrenals (tables 64, 65), fatty appearance of the cut surface of the liver, swelling of the visceral lymph glands and widening of the costochondral junction in some cases (similar to ill. 49). In some of the rats the lungs and other internal organs were fiery red. By microscopical examination the most constant findings were: hyperemia (ill. 27), scattered red blood cells outside the capillaries in the internal organs, degeneration of the renal tubules (ill. 31), deposits of sudanophil droplets between the liver cells, particularly in the Kupffer cells, and slight fatty degeneration of the actual liver cells (ill. 28, 30). By sudan staining, large deposits of sudanophil droplets were usually seen in the adrenal cortex (ill. 40, 42, 43 and table 66). Microscopical examination of the long bones revealed marked hyperemia, periostal and subperiostal hemorrhages, and in some cases hemorrhages in the actual bone, great irregularity of the bone structure with destruction of bone spicules and signs of increased osteoclastic activity, and thinning of the compact bone (ill. 46). In the teeth, marked hyperemia and in some cases hemorrhages were seen in the pulp, as well as vacuoles and deposits of calcium. The odontoblasts were irregularly arranged and appeared degenerated in some cases, and amorphous calcification was observed of the inner part of the dentine (ill. 51, 52, 53, 54, 55).

Chemical analysis of the femurs usually showed no reduction in the calcium or phosphorus contents of the ash, while the ash content of the dry bone in some cases was reduced, and generally showed considerable individual variations.

Similar clinical symptoms and postmortem findings were made in the different types of experimental animals examined.

No direct relation could be detected between the clinical symptoms of hypervitaminosis A and the vitamin A level in the blood, or the vitamin A content of the liver (see page 86).

The Relation of Symptoms to Lesions.

Reduced growth was associated with reduced food intake, as a result of lack appetite, which appeared to be attributed to the action of excess of vitamin A (see page 91).

Of the other clinical symptoms, alopecia, soreness in the skin, swelling of the palpebrae, exophtalmus, stiffness in the limbs, limping, fractures and hemorrhages may also be considered as being attributed to the action of excess of vitamin A.

Alopecia was probably caused by marked hyperemia and hemorrhages around the root sheaths (ill. 38).

Soreness in the skin, often with blood crusts and small hemorrhages was often associated with inflammatory processes in the skin. There was evidence of lowered resistance against infection in hypervitaminotic animals.

Stiffness in the limbs and limping were usually caused by pains in the limbs as a result of fractures or muscular, periostal, subperiostal or osseous hemorrhages.

Roentgenological and histological examinations indicated destruction and absorption of the bone substance, while the remaining bone appeared to contain normal richness of calcium and normal density of bone cells. It would seem natural to consider the bone abnormalities in hypervitaminotic animals in relation to the evidence of increased bone absorption with a large number of osteoclasts, which may possibly be due to a combination of several factors (see p. 189).

Healing of the fractures took place at the same rate as healing of fractures produced in healthy rats for comparison, in spite of the vitamin A being given continuously in unchanged doses, although abnormally rich callus formation was usually observed in the former (see ill. 15, 16, 18).

The hemorrhages in hypervitaminosis A appeared, at least in some cases, to be due to an abnormal permeability of the capillary wall for red blood cells. By the usual staining methods no morphological abnormalities were detected in the vessel walls, however.

The hematuria, which was a frequent finding in hypervitaminotic rats, may well be explained by the presence of erythrocytes in the renal tubules (ill. 33) and in the space of Bowman's capsule (ill. 32) indicating an abnormal permeability of the capsule for red blood cells. On the other hand, the observed deposits of calcium in the degenerated renal tubules (ill. 35) may possibly cause focal lesions of the tubular wall, resulting in hemorrhages into the tubular lumen.

It may be a question whether the small scattered hemorrhages observed in the intestinal wall would be sufficient to explain the positive benzidine test in the feces in hypervitaminotic rats. In some cases, bleeding in the mouth or nose was observed which might give rise to positive benzidine test in the feces.

Fertility was considerably reduced in hypervitaminotic rats, which appeared possibly to be due to absence of libido. No improvement of fertility occurred when hypervitaminotic females were mated with normal males. No significant pathological changes were detected in the genital organs in the male or female rats, except for priapismus in animals with pronounced symptoms, nor were any abortions or abnormal uterine hemorrhages observed (see page 78).

The young from hypervitaminotic mothers were of normal size and weight at birth, and appeared quite normal apart from weakness and dryness of the skin in some cases. The vitamin A content of the liver and of the whole foetus was 4—5 times higher than in normal control foetus at the same stage of development, indicating a considerable transplacental transfer of vitamin A. There was a very high mortality of the

young from hypervitaminotic mothers during the lactation period, during which time symptoms of hypervitaminosis A developed in the young, indicating that the vitamin A transferred through the mothers milk, is sufficient to cause hypervitaminosis A in the young (see page 79).

Hypervitaminosis A and the Action of Other Vitamins.

Additional supply of vitamin B_1 had no influence on the symptoms of hypervitaminosis A. (Experiment 21.)

The condition of hypervitaminosis A did not effect the development of rickets in rats kept on a rachitic diet, nor did it hinder the protective effect of vitamin D given prophylactic to rats kept on a rachitic diet, nor did it prevent the ricket-curing effect of vitamin D in rachitic animals, as judged by roentgenograms, although the ash content of the bones in this case did not rise to normal values. On the other hand excess of vitamin A proved more injurious to rats given a rachitic diet, than to rats given the usual adequate basal diet (see p. 101).

The prothrombin time was prolonged in a number of cases in hypervitaminotic rats, although no direct relationship could be detected between the magnitude of the overdosage with vitamin A and the prothrombin time. Nor was there always any correlation between the prolonged prothrombin time and the incidence of macroscopical hemorrhages. The prolonged prothrombin time was often associated with increased serum colour (table 47), indicating that the observed prolonged clotting time, at least in some cases, might depend on some form of hepatic damage or abnormalities caused by excess of vitamin A, affecting the prothrombin production, — or the formation or excretion of bile acids, thus affecting the absorption of vitamin K from the intestines.

A number of the clinical symptoms and postmortem findings in hypervitaminotic animals were identical with those reported to be observed in human scurvy, such as: muscular weakness, pains in limbs, scattered small hemorrhages apparently due to abnormal capillary permeability, hematuria, intestinal bleeding with diarrhea, subperiostal hematomata, epistaxis, blood extravasations into the muscles, susceptibility to infection, inconstant hypochromic anemia, hepatic and renal damage, widening of the costochondral junction, certain X-ray findings in the long bones, and changes in the teeth such as hyperemia, hemorrhages, vacuoles and deposits of calcium in the pulp, as well as degeneration and disarrangement of the odontoblasts and amorphous calcification of the inner part of the dentine (ill. 51, 52, 53, 54, 55). Even the microscopical picture of the long bones may to some extent resemble that of scurvy (ill. 46). In the majority of cases, abnormally low ascorbic acid contents were found in the serum, adrenals and liver (tables 48, 49, 50, 51).

Furthermore the clinical picture and the postmortem findings in guinea pigs given excess of vitamin A, and in those given a scorbutic

diet, were very similar, and similar gross doses of vitamin A proved more toxic to guinea pigs given a scorbutic diet, than in those given the usual adequate basal diet (see p. 170).

Although prolonged administration of excess of vitamin A in experimental animals produced a condition which largely resembled scurvy, abnormality of the vitamin C metabolism cannot in any way be considered as the sole causitive factor in hypervitaminosis A. Furthermore, large doses of vitamin C did not offer any significant protection against the injurious effect of massive doses of vitamin A, although additional supply of vitamin C had a beneficial effect against moderate excess of vitamin A (see pages 121, 171).

Pathogenesis.

Several of the clinical symptoms in hypervitaminosis A have been found to correspond to histological changes attributed to the toxic effect of excess of vitamin A. The mechanism of this toxic effect on the cells remains obscure, however, and should be subject to further investigations.

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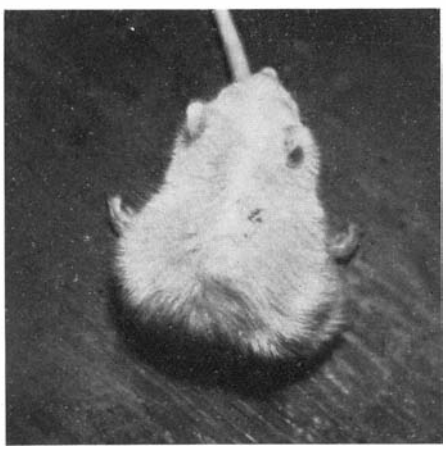
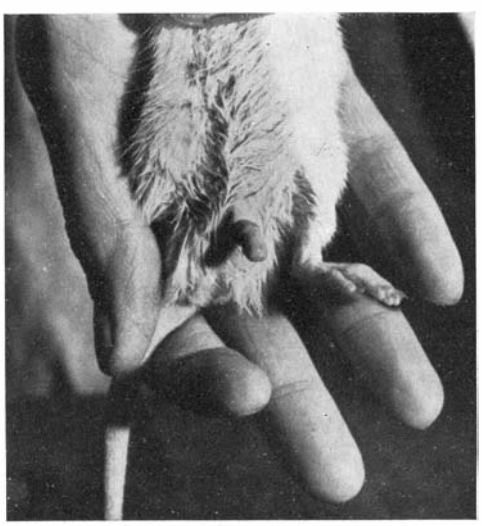
PLATES

Pl. II.

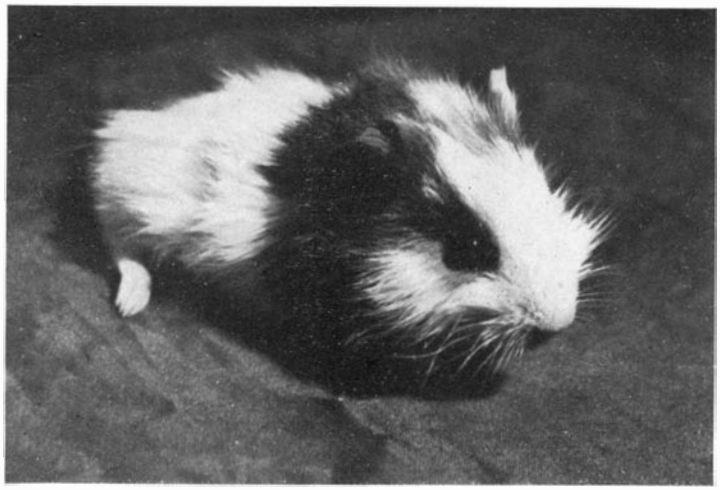
Ill. 4. Scruffiness, exophtalmus and fractures with bone deformities in rachitic rat given excess of vit. A. (Experiment 26, p. 97).



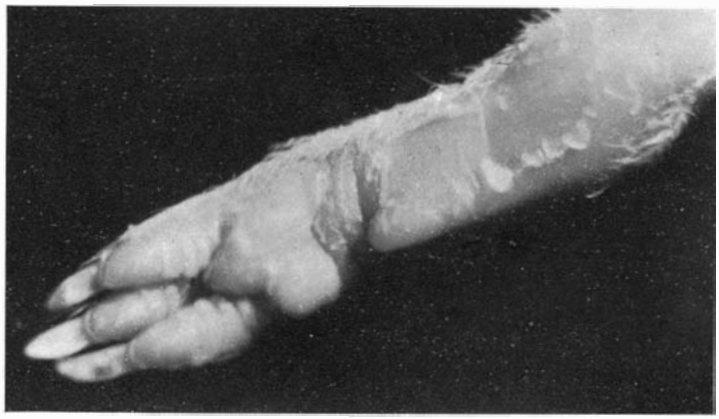
Ill. 5. Abnormal posture, with marked bending of the spine, and "paralysis" of both hind legs, without any fractures or hemorrhages being detected in the lower extremities in adult rat given 625 I. U. vit. A/g body weight daily for 24 days. (Experiment 20, p. 70).



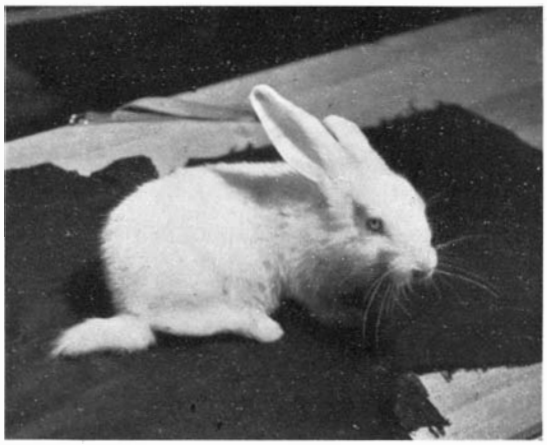
Ill. 6. Priapismus in rachitic rat given 50,000 I. U. vit. A. daily (Experiment 26, p. 97).



Ill. 7. Guinea pig no. 13 given 370 I. U. vit. A/g body weight daily. Fracture of right hind leg on the 6th day.



Ill. 8. Peeling of the skin in hypervitaminotic guinea pig no. 31 given 178 I. U. vit. A/g body weight daily for 43 days.



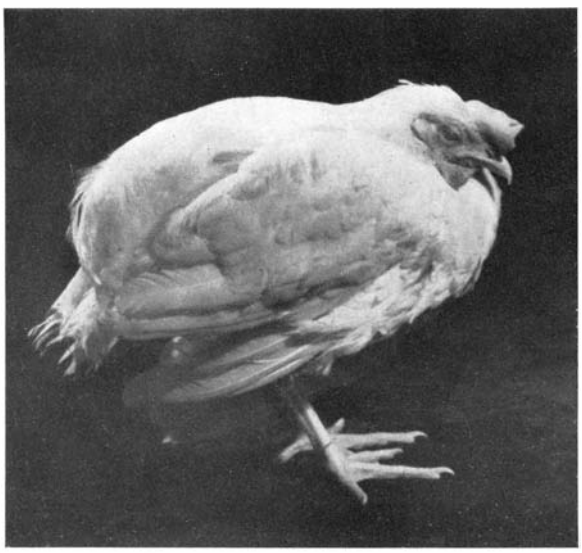
Ill. 9. Young rabbit given 244 I. U. vit. A/g body weight daily (Experiment 51, p. 174). Fracture of right hind leg at the end of 20 days (see also ill. 25, 26 and 44).

Pl. IV.

a.

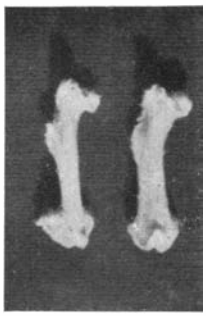
b.

Ill. 10. a. Normal control cockerel.
b. Cockerel given 235—286 I. U. vit. A/g body weight daily for 10 days (Experiment 53). Pale, sore and drooping comb, soreness and swelling of the eyes.

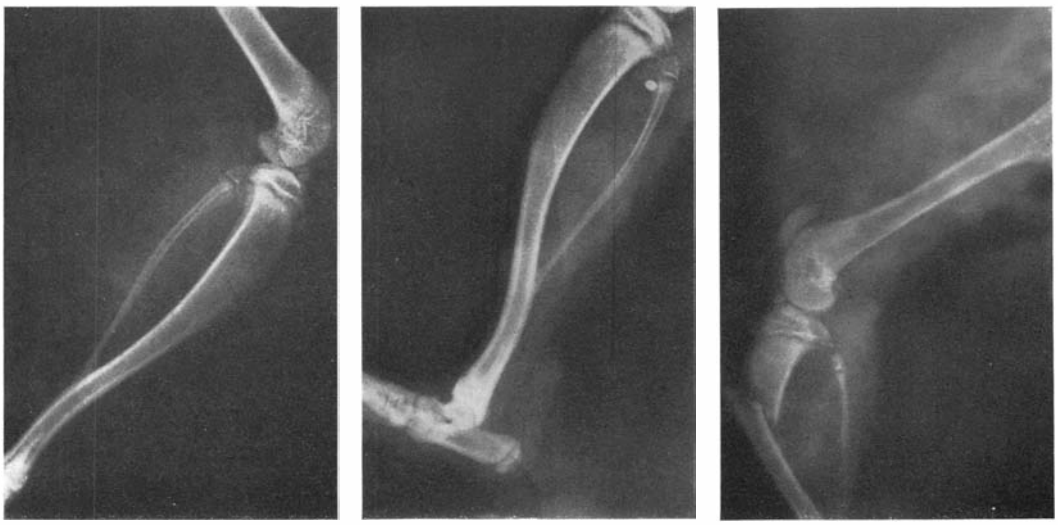


Ill. 11. Hypervitaminotic cockerel (Experiment 53), showing drooping tail feathers and comb, and soreness around eyes.

Pl. V.

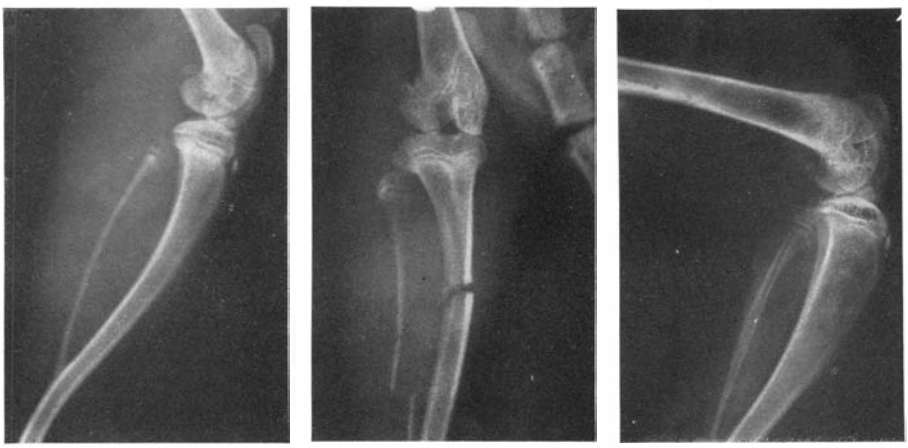


Ill. 12. Femur of adult hypervitaminotic rat (left), compared with the femur of a normal control rat of the same age (right) showing the difference in diameter. (Experiment 8).



a. b. c.

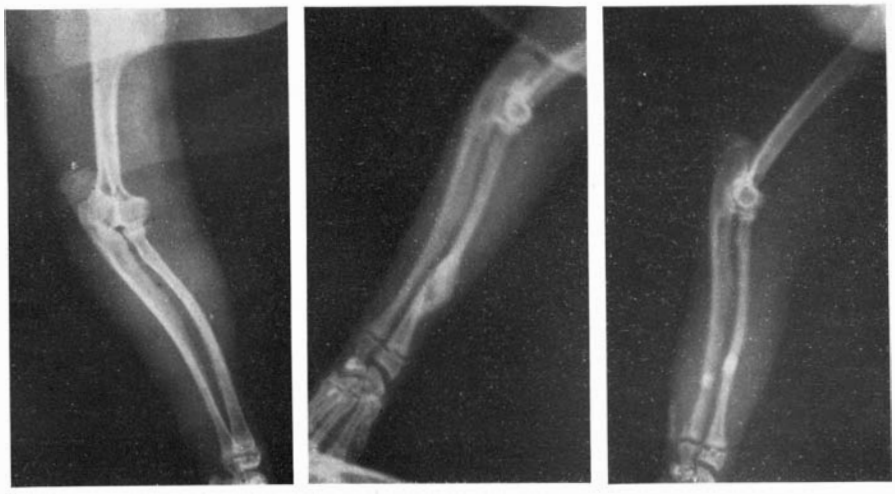
Ill. 13. Roentgenograms showing the bones of one of the hind legs in normal control rat (a), compared with rat of the same age given liver oil freed of its vitamin A content (b) (Exp. 5), and rat of the same age given the same amount of the same oil containing all its vitamin A during a similar period (c). In the first two cases no abnormalities are detected, while in the third case there is thinning of the bone shafts and fracture of the tibia.



a. b. c.

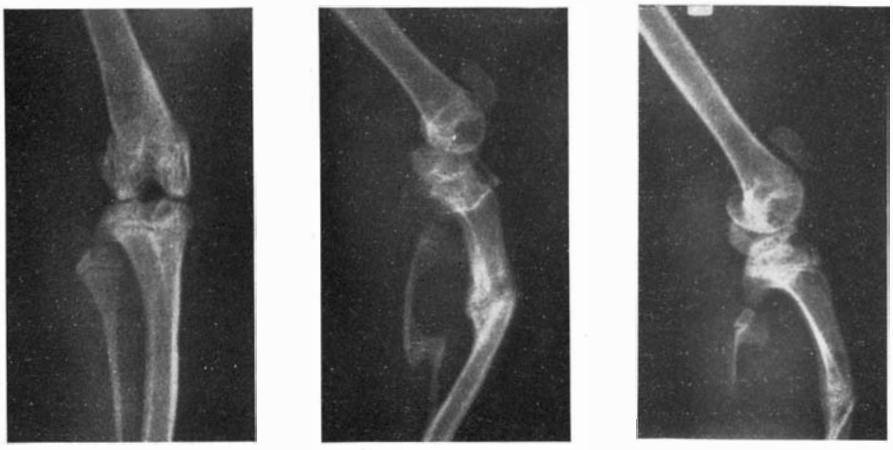
Ill. 14. Roentgenograms of left hind leg in male rat given 50,000 I. U. vit. A daily (500—250 I. U. vit. A/g body weight) at the end of 28 days (a) and 60 days (b) (Exp. 17), compared with normal control rat of the same age at the end of 30 days (c), showing abnormal thinning of the bone shafts in the hypervitaminotic animal (a) followed by fractures of the tibia and fibula (b).

Pl. VI.



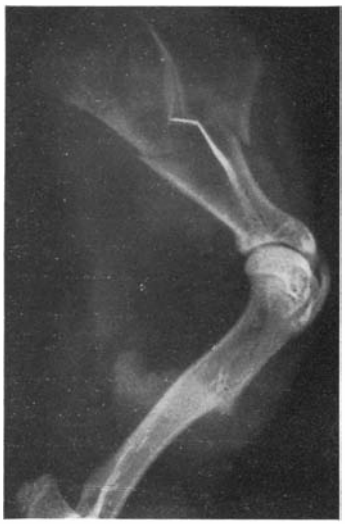
a. b. c.

Ill. 15. Roentgenograms of left fore leg in the same rat as shown in ill. 14 a, and b, at the end of 10 (a), 28 (b) and 62 (c) days from the beginning of the experiment, showing healing of fractures in spite of the excess of vitamin A being given continuously in unchanged doses.

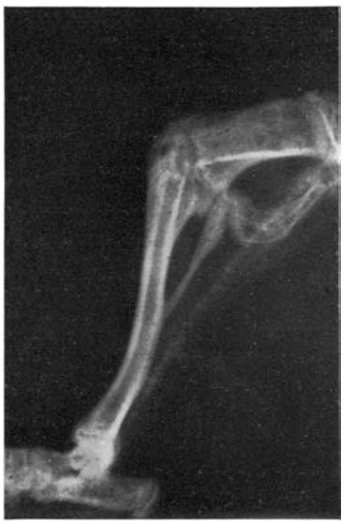


a. b. c.

Ill. 16. Roentgenograms of right hind leg of female rat given 50,000 I. U. vit. A daily (500—250 I. U. vit. A/g body weight) at the end of 53 days (a), showing abnormal thinning of the bone shafts, and at the end of 74 (b) and 106 days (c), showing healing of fractures of the tibia and fibula. (Experiment 17, p. 63).



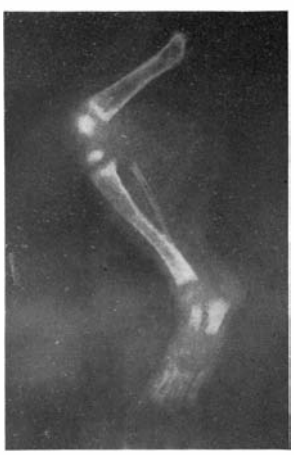
Ill. 17.



Ill. 18.

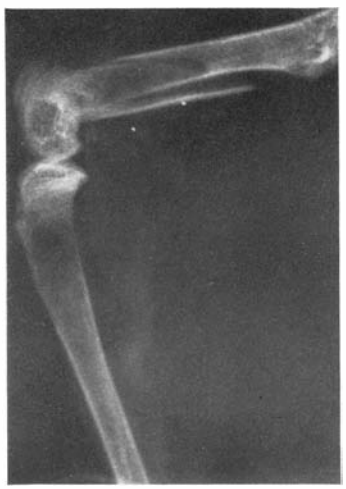
Ill. 17. Fracture of scapula in adult hypervitaminotic rat (390 I. U. vit. A/g body weight daily) at the end of 12 days. (Experiment 8, p. 41).

Ill. 18. Healing of fractures in hypervitaminotic rat with marked callus formation. (Experiment 21, p. 92).



Ill. 19. Roentgenogram of right hind leg in young rat from hypervitaminotic mother, showing thinning of the cortical shadow, a broad dense shadow around the centres of ossification, and irregular, intensely calcified zones at the epiphyseal ends of the tibia (see p. 76).

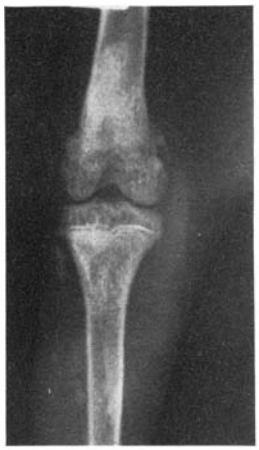
Pl. VIII.



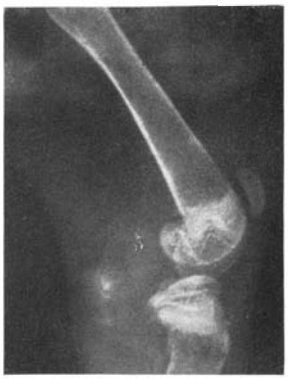
a.

b.

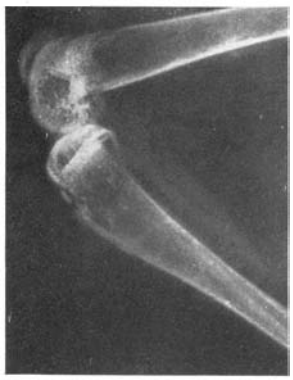
Ill. 20. Roentgenograms of scorbutic (a) and hypervitaminotic (b) guinea pigs, showing thinning of bone shafts and cortical shadows, and lines of dense calcification at the epiphyseal border of the metaphyses in both cases. (guinea pigs nos. 21 and 24).



Ill. 21. Roentgenogram of hypervitaminotic guinea pig showing abnormally thin bone shafts, particularly the fibula, which has practically disappeared (guinea pig no. 31).

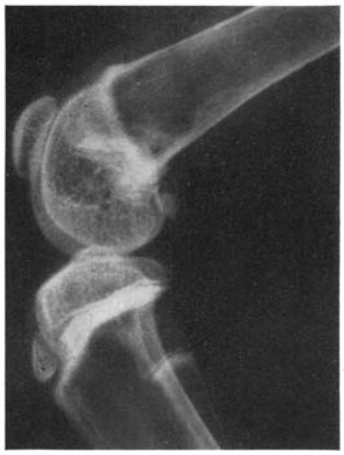


Ill. 22.

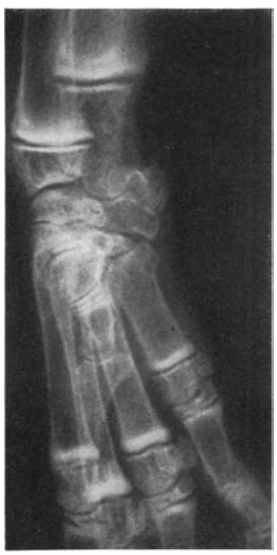


Ill. 23.

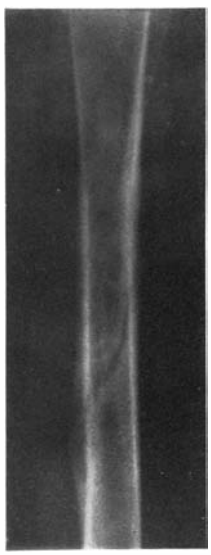
Roentgenograms of young guinea pigs nos. 13 (370 I. U. vit. A/g body weight daily for 12 days (ill. 22)) and no. 28 (290—390 I. U. vit. A/g body weight + 50 mg ascorbic acid daily for 11 days (ill. 23)) showing thinning of cortical shadow, lines of dense calcification at the epiphyseal border of the metaphyses and fracture of the proximal end of the tibia in both cases.



Ill. 24. Roentgenogram of knee joint in rabbit given 356 I. U. vit. A/g body weight daily for 10 days, showing thinning of cortical shadows and lines of dense calcification at the epiphyseal borders of the metaphyses. (Experiment 51).



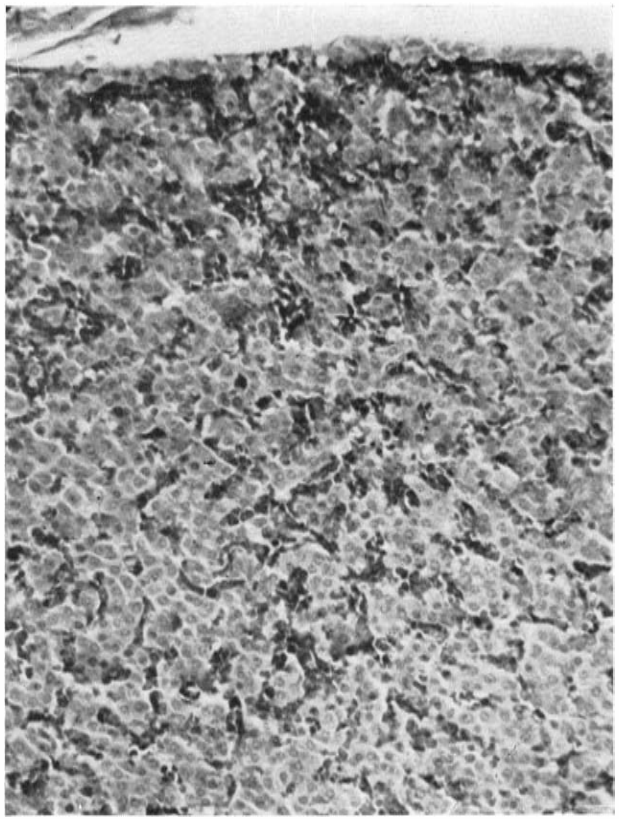
Ill. 25.



Ill. 26.

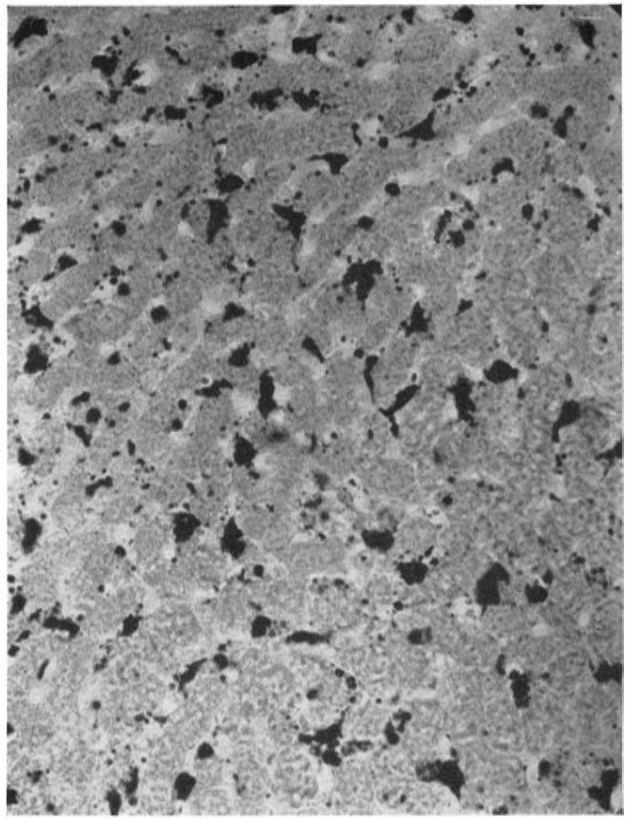
Ill. 25. Roentgenogram of fore leg in rabbit given 244 I. U. vit. A/g body weight daily for 30 days, showing similar findings as in ill. 24.

Ill. 26. Roentgenogram of right tibia of the same rabbit as ill. 9, showing oblique fracture and shadow indicating periostal callus.



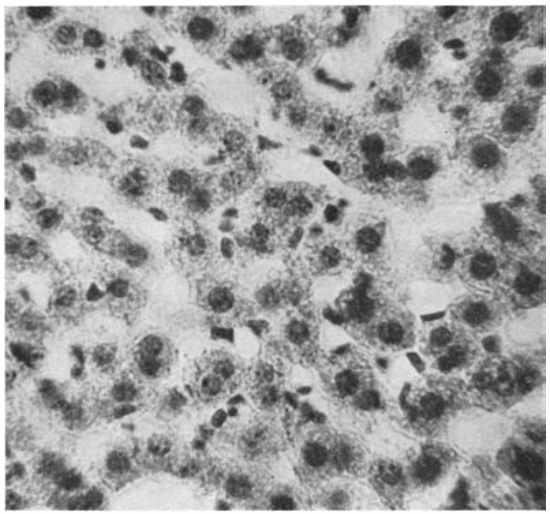
Hematoxylin-eosin staining. × 240

Ill. 27. Liver of a young rat from hypervitaminotic mother, showing marked hyperemia (see p. 76).



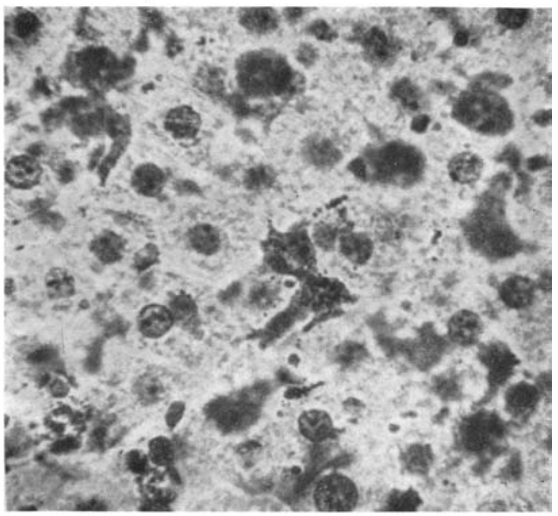
× 200

Ill. 28. Liver, stained by sudan III from adult male rat given 250—390 I. U. vit. A/g body weight daily, showing massive deposits of sudanophil droplets between the liver cells, especially in the Kupffer cells. (Experiment 8, p. 41).



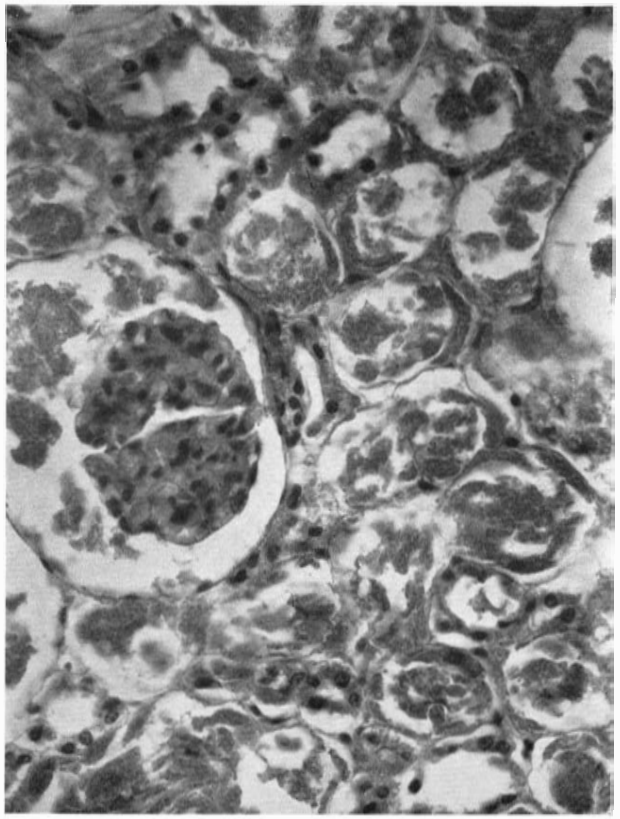
× 330

Ill. 29. Liver from normal control rat stained by sudan III, showing no sudanophil deposits. (Experiment 5, p. 35).



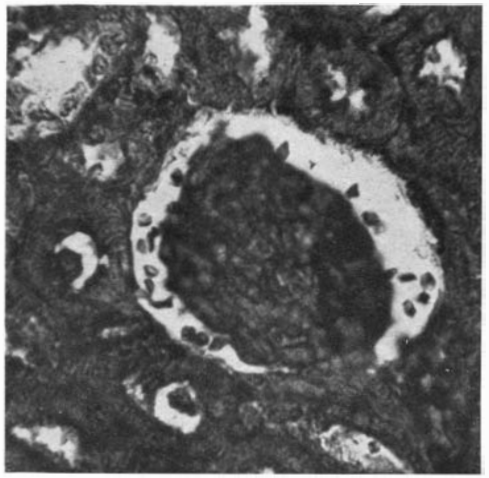
× 540

Ill. 30. Liver, stained by sudan III, from adult female rat given 200 I. U. vit. A/g body weight daily for 51 days, showing deposits of large sudanophil droplets between the liver cells, especially in the Kupffer cells, which were swollen. (Experiment 19).



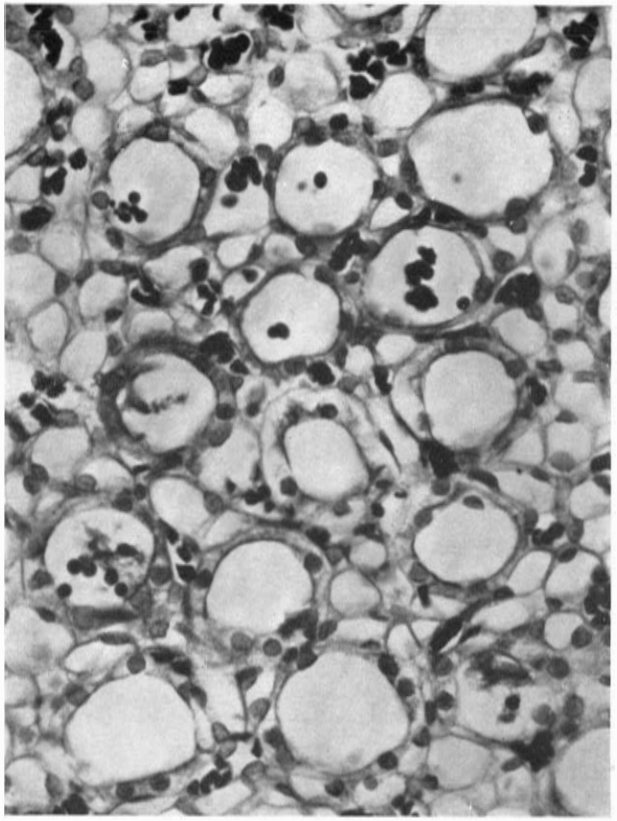
Hematoxylin-eosin staining. × 390

Ill. 31. Kidney from rat given 500—250 I. U. vit. A/g body weight daily for 119 days, showing degeneration and necrosis of convoluted tubules (Experiment 17, p. 62).



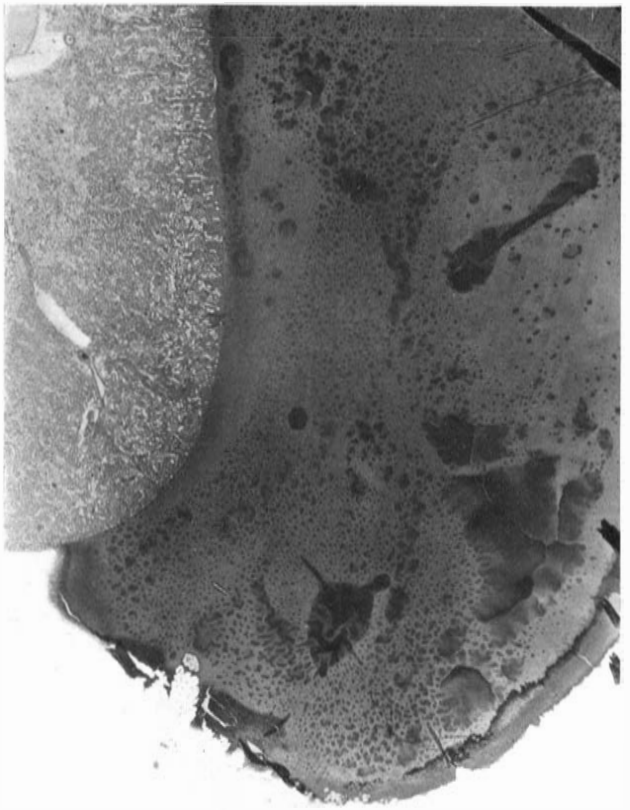
Mallory staining. × 420

Ill. 32. Kidney from rat given 52,500 I. U. vit. A daily for 11 days, showing red blood cells in the space of Bowman's capsule. (Experiment 2, p. 27).



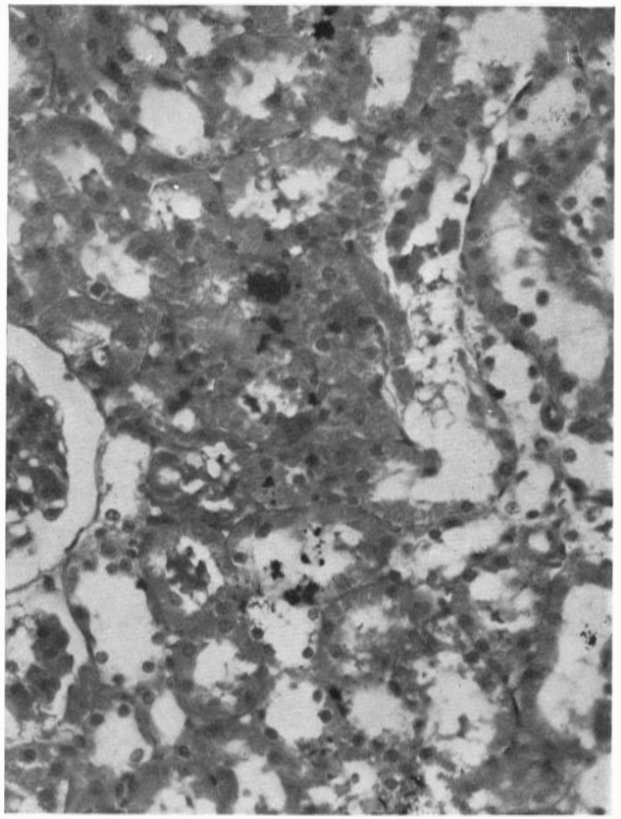
Hematoxylin-eosin staining. × 500

Ill. 33. Kidney from same rat as shown in ill. 32 showing red blood cells in collecting tubules.



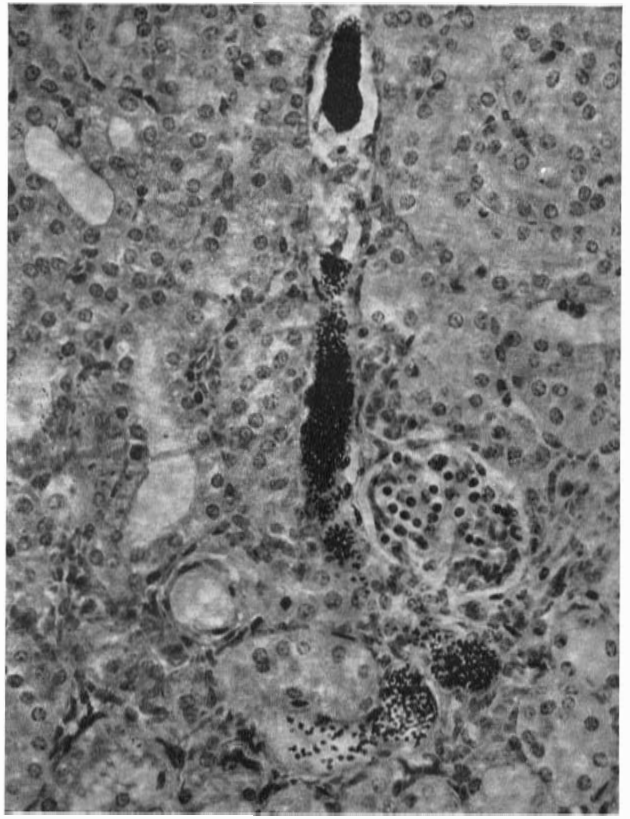
Hematoxylin-eosin staining. × 11

Ill. 34. Large hematoma around the kidney in rat which suddenly died at the end of 78 days, after having received 525—188 I. U. vit. A/g body weight daily.
(Experiment 15, p. 54).



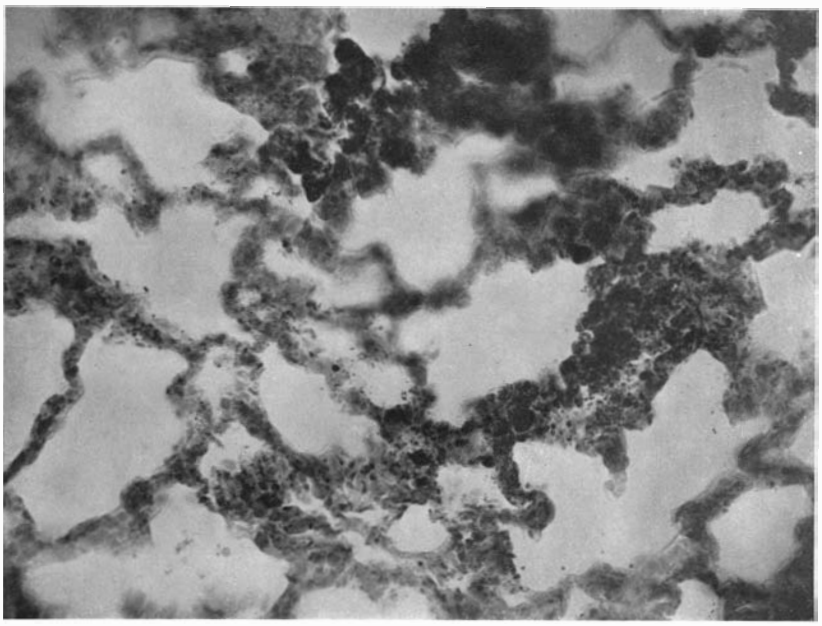
Kossa's staining. × 330

Ill. 35. Kidney from adult rat given 250—390 I. U. vit. A/g body weight daily for 46 days, showing scattered deposits of calcium in the convoluted tubules.
(Experiment 8, p. 41).



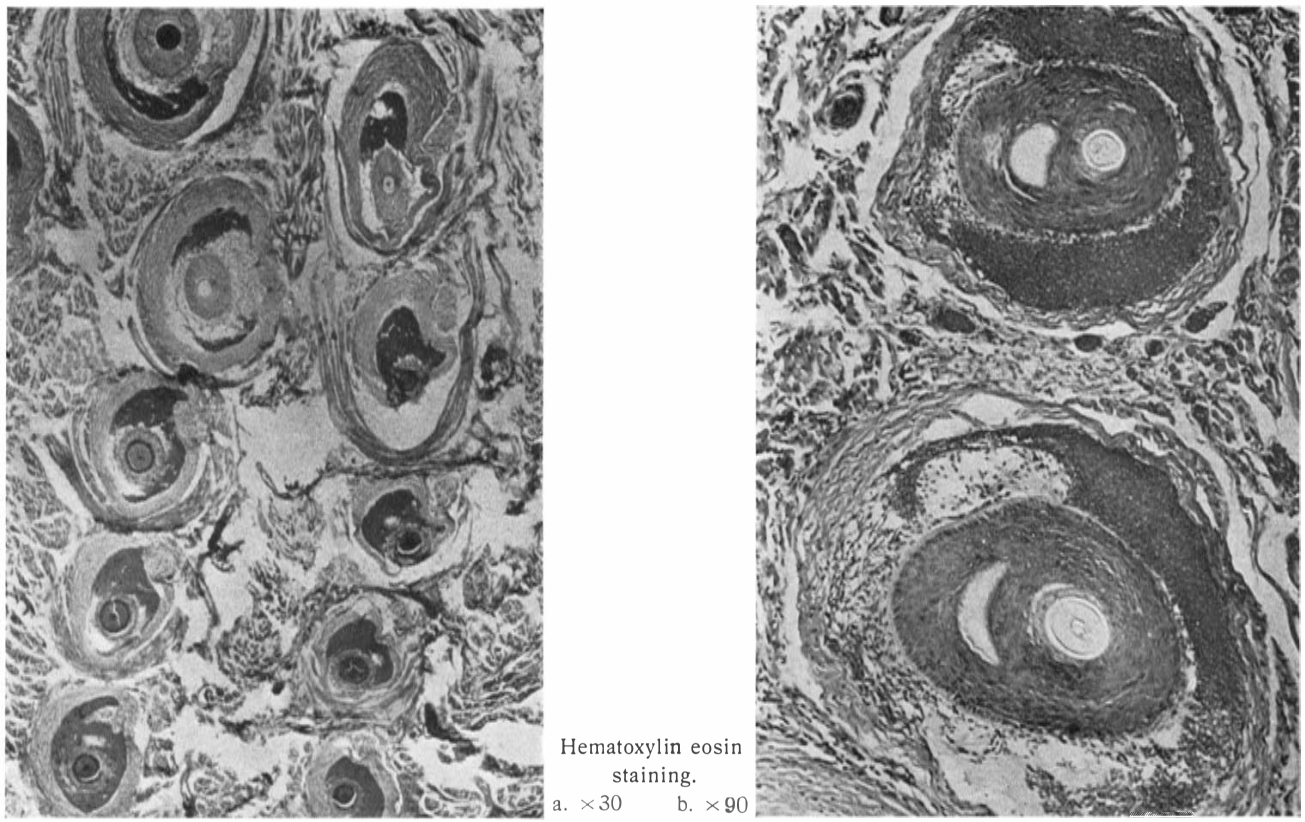
Kossa's staining. × 330

Ill. 36. Kidney from guinea pig no. 27 given 300 I. U. vit. A/g body weight daily for 8 days, showing deposits of calcium.



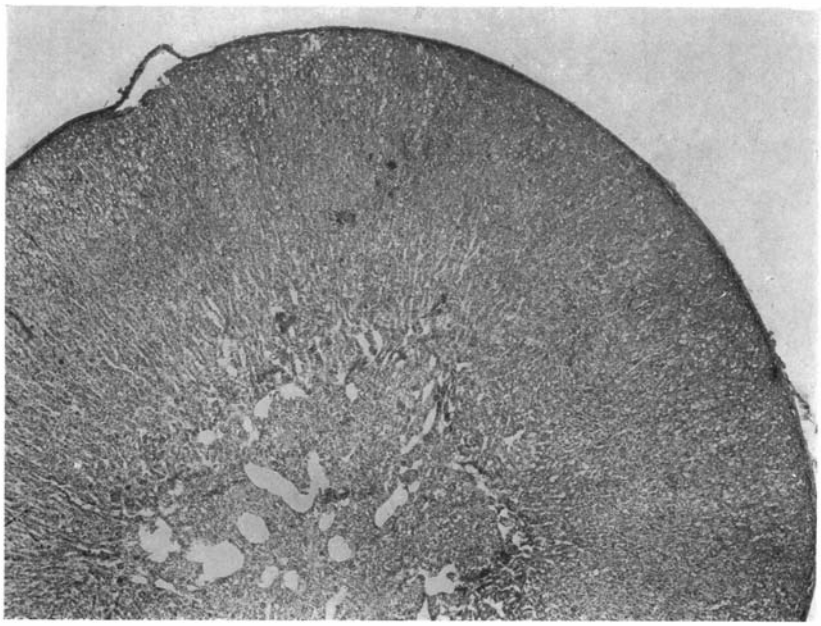
× 200

Ill. 37. Lung, stained by sudan III, from adult rat given 250—390 I. U. vit. A/g body weight daily for 46 days, showing sudanophil deposits in the alveolar wall. (Experiment 8, p. 42).



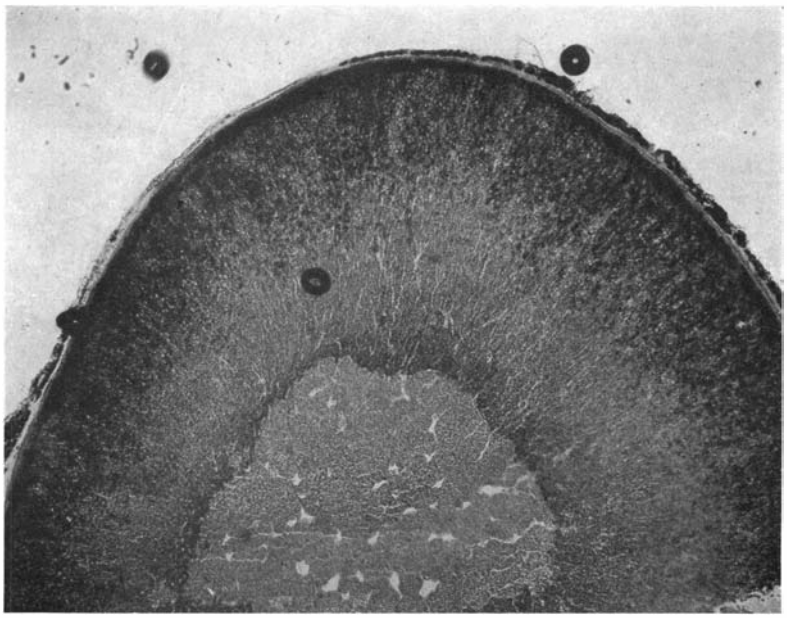
Hematoxylin eosin staining.
a. ×30 b. ×90

Ill. 38. Microphotographs of slides taken from the skin at the places of alopecia in a rat given 52,500 I. U. vit. A daily for 11 days (Experiment 2, p. 27), showing marked hyperemia and hemorrhages around the root sheaths of the hair (in the dermic coat).



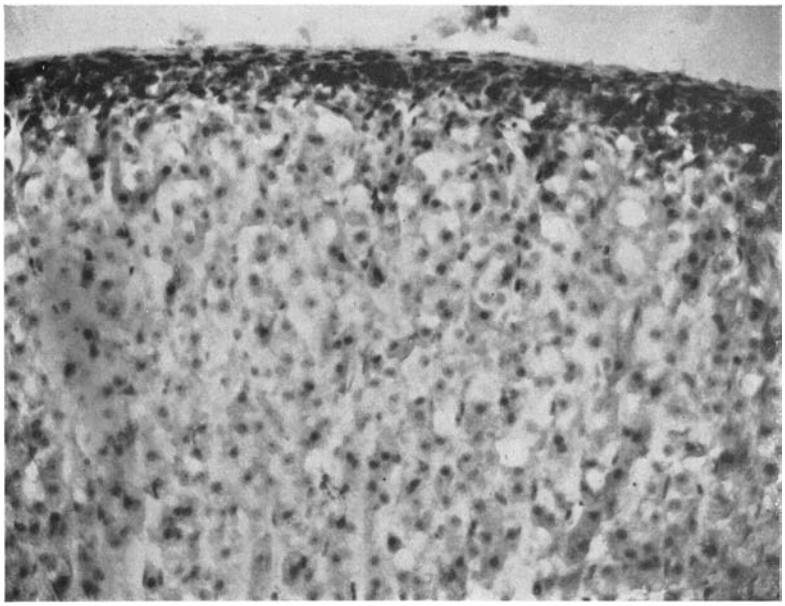
× 30

Ill. 39. Adrenal of normal adult female rat stained by sudan III. (Experiment 5, p. 35).



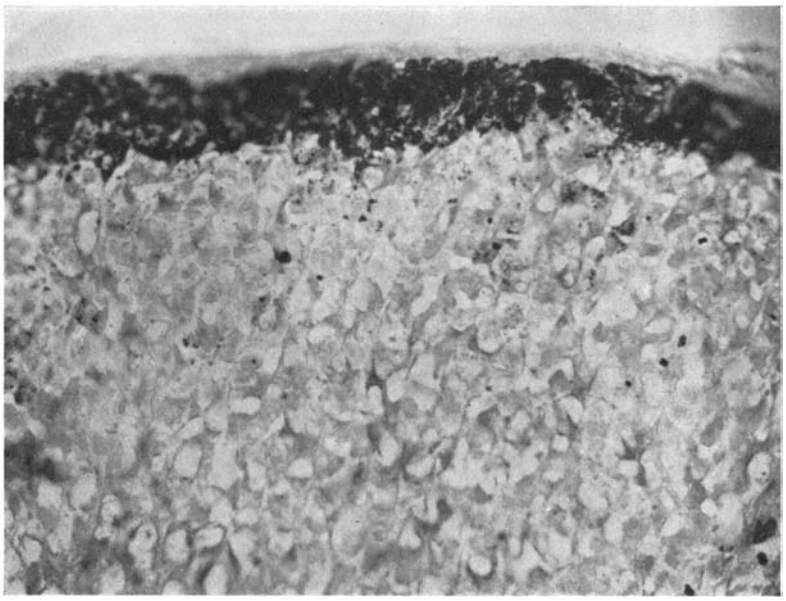
× 30

Ill. 40. Adrenal of adult male hypervitaminotic rat (250—390 I. U. vit. A/g body weight daily for 46 days) stained by sudan III, showing dense sudanophil deposits in the cortex. (Experiment 8, p. 41).



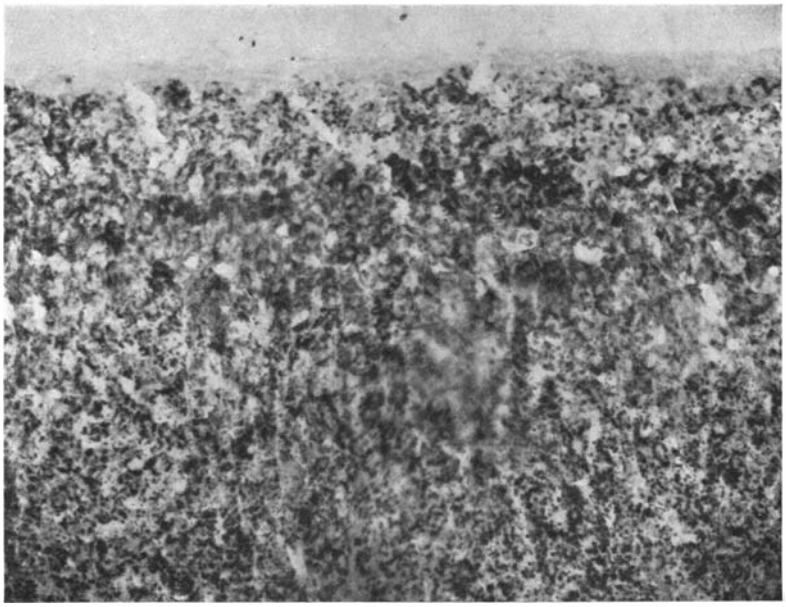
× 200

Ill. 41. Adrenal cortex of normal adult female rat stained by sudan III, (same as ill. 39).



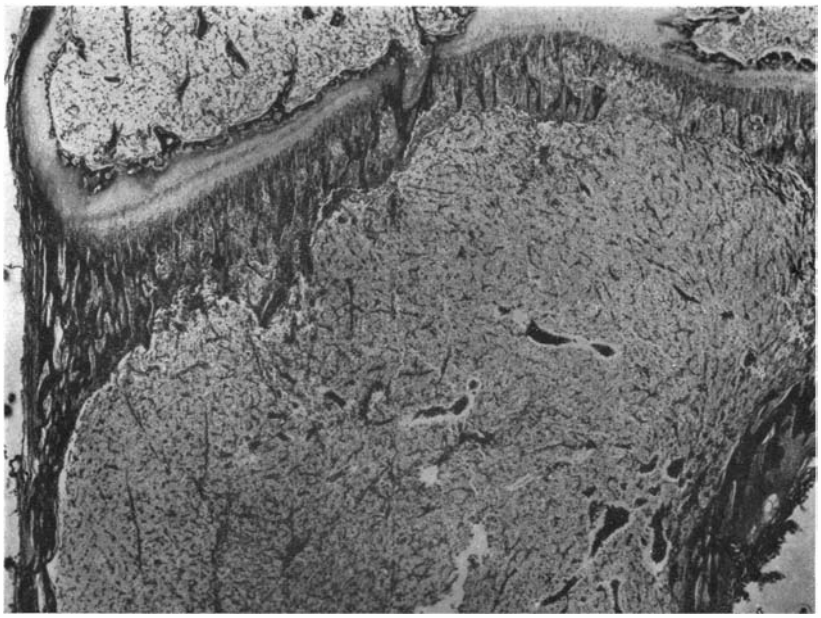
× 200

Ill. 42. Adrenal cortex of adult male hypervitaminotic rat (260—480 I. U. vit. A/g body weight daily for 44 days), stained by sudan III, showing dense sudanophil deposits in the zona glomerulosa. (Experiment 8, p. 40).



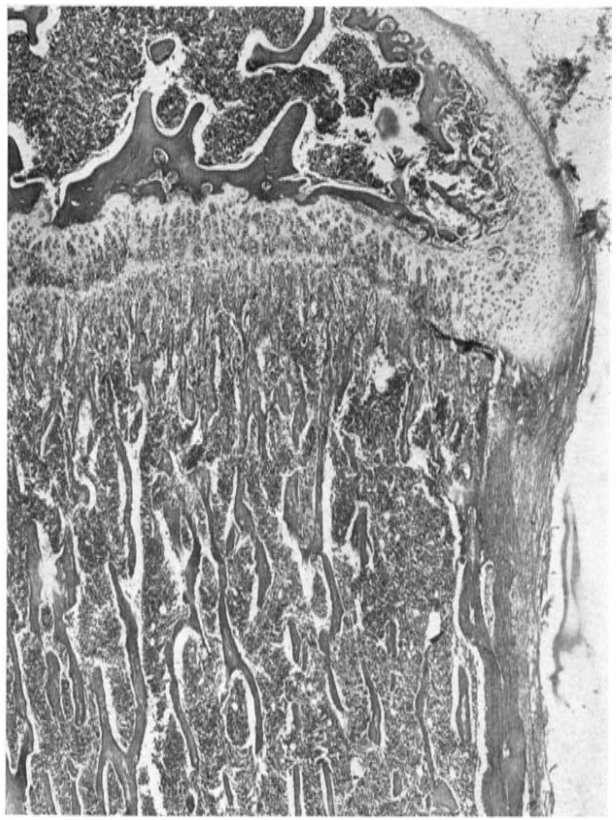
× 200

Ill. 43. Adrenal cortex of adult male hypervitaminotic rat (625 I. U. vit. A/g body weight daily for 46 days), stained by sudan III, showing dense sudanophil deposits in the zona fasciculata. (Experiment 20, p. 70).



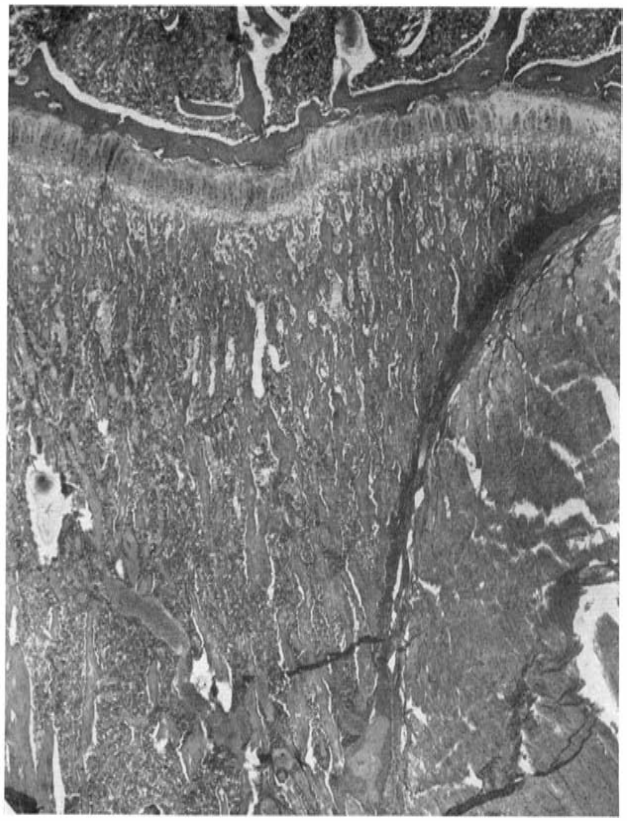
Hematoxylin-eosin staining. × 15

Ill. 44. Tibia from young rabbit (same as ill. 9, 25 and 26) given 244 I. U. vit. A/g body weight daily for 30 days, showing irregularity and destruction of bone spicules.



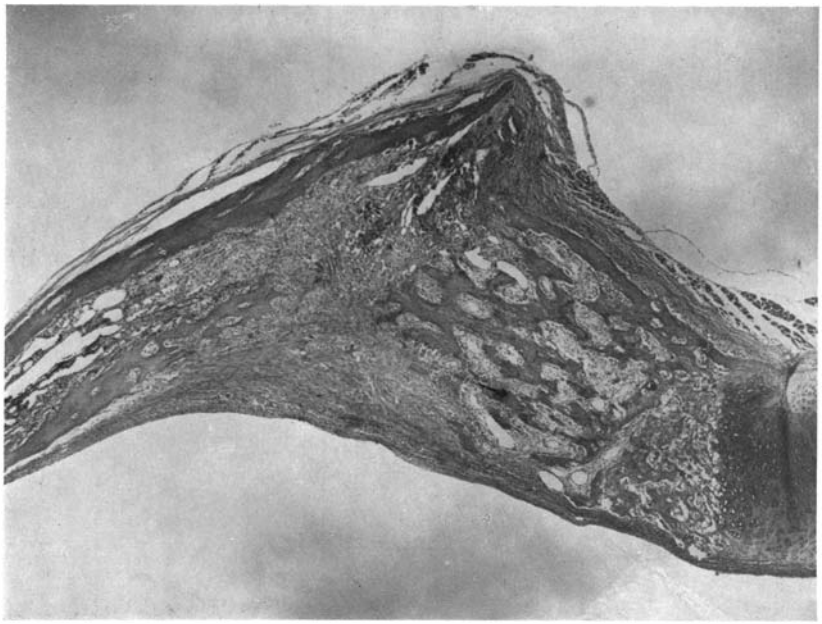
Hematoxylin-eosin staining. × 27

Ill. 45. Tibia from young rat given 95 I. U. vit. A/g body weight daily for 18 days, showing no significant pathological conditions.



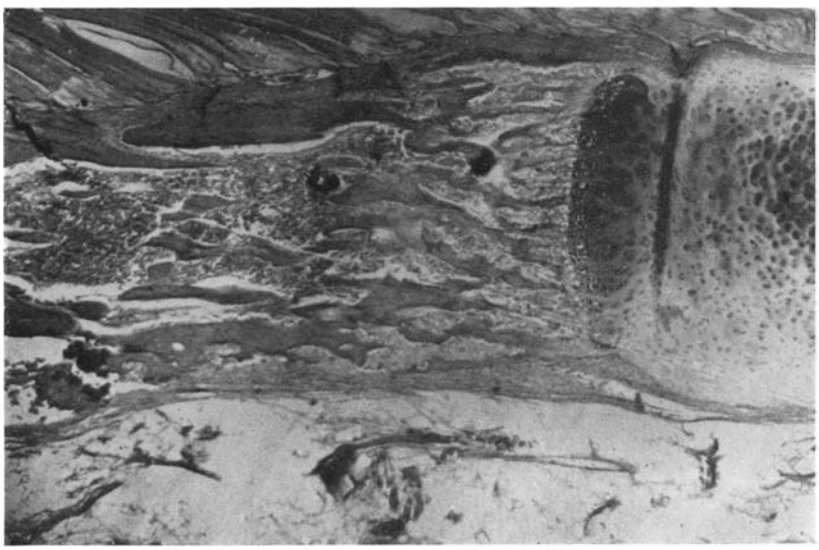
Hematoxylin-eosin staining. × 27

Ill. 46. Tibia from rat of same age as Ill. 45, given 500—830 I. U. vit. A/g body weight daily for 27 days, showing narrow epiphyseal line, thinning and irregularity of compact bone, and subperiostal hemorrhage. (Exp. 1, p. 25).



Hematoxylin-eosin staining. × 27

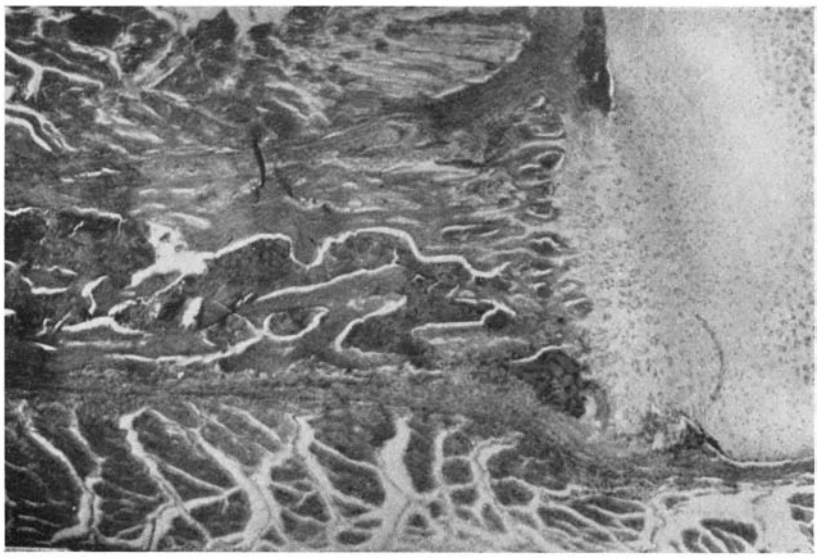
Ill. 47. Fracture of rib in guinea pig no. 15 given 110—145 I. U. vit. A/g body weight daily for 17 days. (Experiment 44).



Hematoxylin-eosin staining. × 30

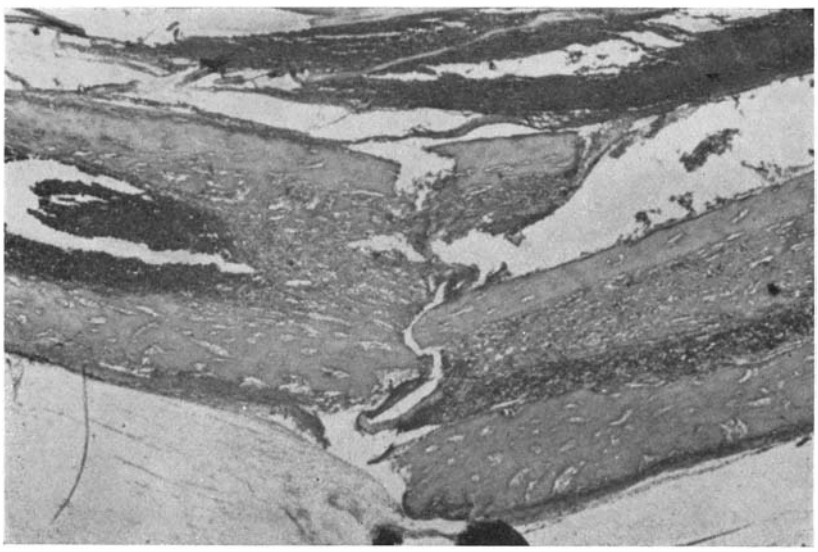
Ill. 48. Costochondral junction in guinea pig no. 36, given 175 I. U. vit. A/g body weight + 50 mg ascorbic acid daily for 65 days, showing irregularity of bone structure.

Pl. XXII.



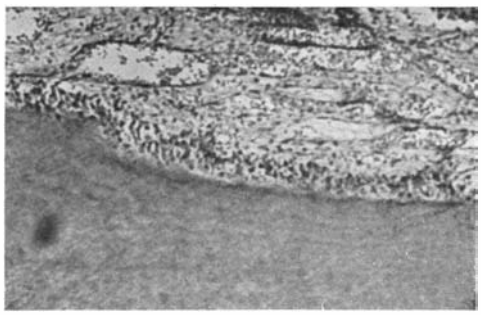
Hematoxylin-eosin staining. × 30

Ill. 49. Costochondral junction of guinea pig no. 34, given 280—160 I. U vit. A/g body weight daily for 61 days, showing widening and deformity of the junction, great irregularity of bone structure with destruction of bone spicules, and thinning of the compact bone, as well as marked hyperemia and periostal and subperiostal hemorrhages.



Hematoxylin-eosin staining. × 35

Ill. 50. Fracture of tibia in rat given vit. B_1 in addition to excess of vit. A (570 I. U. vit. A/g body weight daily) for 21 days (Exp. 21).

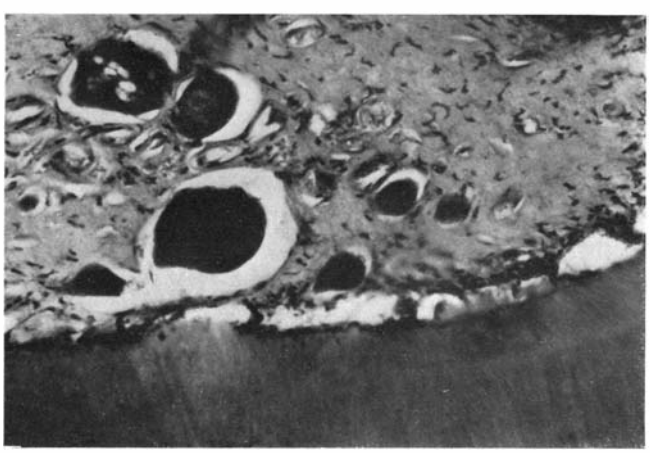


Hematoxylin-eosin staining. × 100

Ill. 51. Microphotograph of incisor tooth from young rat given excess of vit. A (50,000 I. U. vit. A daily) in addition to rachitic diet for 11 days, showing irregularity of the odontoblasts. (Experiment 24, p. 96).

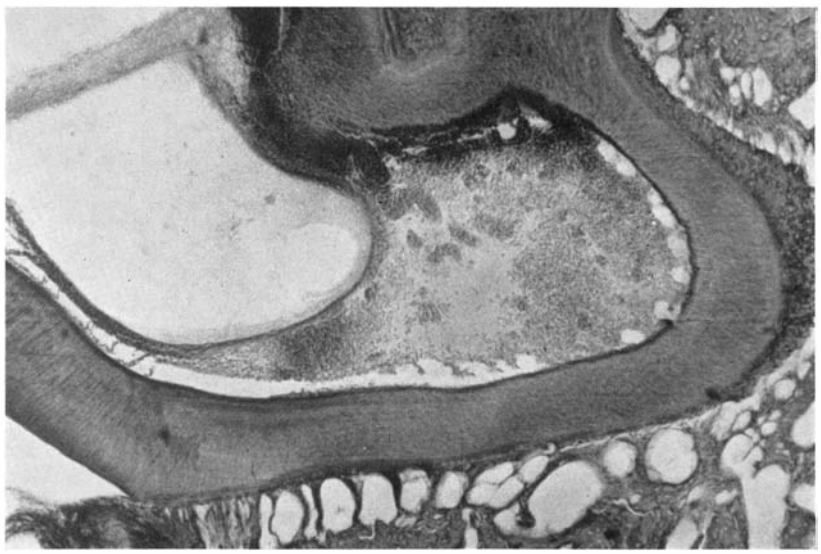
Hematoxylin-eosin staining. × 35

Ill. 52. Microphotograph of incisor tooth from rat given 52,500 I. U. vit. A daily for 11 days, showing marked hyperemia in the pulp. (Exp. 2, p. 27).



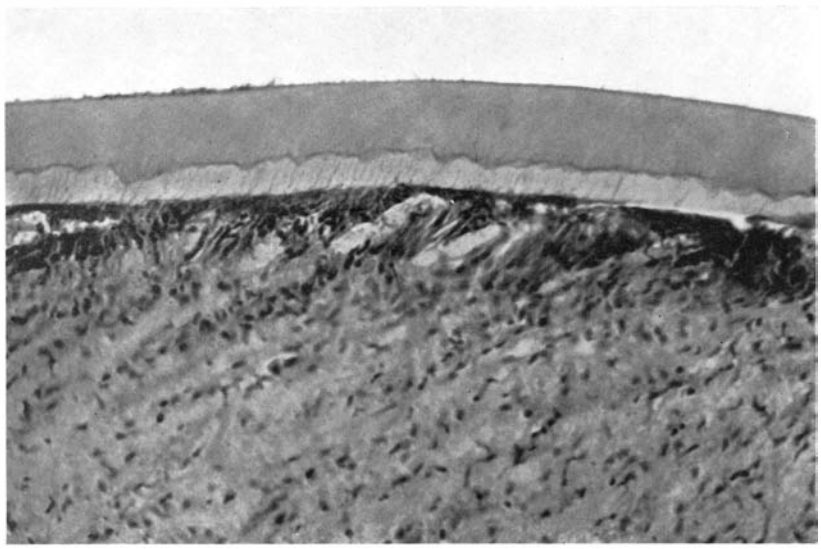
Hematoxylin-eosin staining. × 250

Ill. 53. Microphotograph of tooth from young hypervitaminotic rat, showing degeneration of the odontoblasts and the pulp cells, amorphous calcification of the inner part of the dentine, and deposits of calcium in the pulp.



× 70

Ill. 54. Microphotograph of tooth in young hypervitaminotic rat, showing vacuoles and hemorrhage in the pulp and amorphous calcification of the inner part of the dentine.



Ill. 55. Microphotograph of incisor tooth from young hypervitaminotic rat, showing degeneration of the pulp cells and the odontoblasts and changes in the dentine.

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