REFERENCES

Association of Official Agricultural Chemists (1940). *Official and Tentative Methods of Analysis*, 5th ed. Washington: Association of Official Agricultural Chemists.
Bailey, C. H. (1944). *The Constituents of Wheat and Wheat Products*. New York: Reinhold Publishing Corporation.
Barton-Wright, E. C. (1944). *Biochem. J.* **38**, 314.
Barton-Wright, E. C. & Booth, R. G. (1943). *Biochem. J.* **37**, 25.
Booth, R. G. (1942). *Analyst*, **67**, 162.
Booth, R. G., Carter, R. H., Jones, C. R. & Moran, T. (1941*a*). *Chem. Ind.* **19**, 903.
Booth, R. G., Carter, R. H., Jones, C. R. & Moran, T. (1941*b*). *Chem. Ind.* **20**, 245.
Briggs, A. P. (1922). *J. biol. Chem.* **53**, 13.
Cowling, H. & Benne, E. J. (1942). *J. Ass. off. agric. Chem., Wash.*, **25**, 562.
von Fellenberg, T. (1918). *Biochem. Z.* **88**, 323.
Fertilisers and Feeding Stuffs Regulations (1932). Statutory Rules and Orders, No. 658. London: H.M. Stationery Office.
Fox, F. W. & Golberg, L. (1944). *Publ. S. Afr. Inst. med. Res.* no. 46, **9**, 123.
Greer, E. N., Mounfield, J. D. & Pringle, W. J. S. (1942). *Analyst*, **67**, 352.
Herd, C. W. & Amos, A. J. (1930). *Cereal Chem.* **7**, 251.
Hinton, J. J. C. (1944). *Biochem. J.* **38**, 214.
Kent, N. L., Simpson, A. G., Jones, C. R. & Moran, T. (1944). *High Vitamin Flour*. London: Ministry of Food.
Kent-Jones, D. W. (1939). *Modern Cereal Chemistry*, 3rd ed. Liverpool: Northern Publishing Co.
Krebs, H. A. & Mellanby, K. (1942). *Lancet*, **1**, 319.
Lane, J. H. & Eynon, L. (1923). *J. Soc. chem. Ind., Lond.*, **42**, 32T.
von Lieberman, L. & Szekely, S. (1898). *Pflüg. Arch. ges. Physiol.* **72**, 360.
McCance, R. A. & Lawrence, R. D. (1929). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 135.
McCance, R. A. & Shipp, H. L. (1933). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 187.
McCance, R. A. & Widdowson, E. M. (1935). *Biochem. J.* **29**, 2694.
McCance, R. A. & Widdowson, E. M. (1940). *Spec. Rep. Ser. Med. Res. Coun., Lond.* Publication delayed for security reasons.
McCance, R. A. & Widdowson, E. M. (1942). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 235.
McCance, R. A., Widdowson, E. M. & Shackleton, L. R. B. (1936). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 213.
Macrae, T. F., Hutchinson, J. O., Irwin, J. O., Bacon, J. S. D. & McDougall, E. J. (1942). *J. Hyg., Camb.*, **42**, 423.
Martin, C. J. & Robison, R. (1922). *Biochem. J.* **16**, 407.
Ministry of Food, Scientific Adviser's Division (1942*a*). *Nature, Lond.*, **149**, 460.
Ministry of Food, Scientific Adviser's Division (1942*b*). *Nature, Lond.*, **150**, 538.
Ministry of Food, Scientific Adviser's Division (1943). *Nature, Lond.*, **151**, 629.
Ministry of Food, Scientific Adviser's Division (1944*a*). *Nature, Lond.*, **153**, 154.
Ministry of Food, Scientific Adviser's Division (1944*b*). *Nature, Lond.*, **154**, 582.
Moran, T. (1945). *Nature, Lond.*, **155**, 205.
Moran, T. & Drummond, J. (1945). *Lancet*, **1**, 698.
Moran, T. & Pace, J. (1942). *Nature, Lond.*, **150**, 224.
Newman, L. F., Robinson, G. W., Halnan, E. T. & Neville, H. A. D. (1912). *J. Hyg., Camb.*, **12**, 119.
Nicholls, J. R., Booth, R. G., Kent-Jones, D. W., Amos, A. J. & Ward, H. H. (1942). *Analyst*, **67**, 15.
Pringle, W. J. S. & Moran, T. (1942). *J. Soc. chem. Ind., Lond.*, **61**, 108.
Royal Society, Food (War) Committee (1918). *Report on the Digestibility of Breads.*
Rubner, M. (1916). *Arch. Anat. Physiol., Lpz.*, Physiol. Abt. p. 61.
Sherman, H. C. (1937). *The Chemistry of Food and Nutrition*, 5th ed. New York: Macmillan.
Snyder, H. (1901). *Bull. U.S. Off. Exp. Stas.* no. 101. Cited by Macrae *et al.* (1942).
Snyder, H. (1905). *Bull. U.S. Off. Exp. Stas.* no. 156. Cited by Macrae *et al.* (1942).
Sullivan, B. & Near, C. (1927). *Industr. Engng Chem.* **19**, 498.
Teller, G. L. (1896). *Bull. Ark. agric. Exp. Sta.* no. 42. Cited by Kent-Jones, D. W. (1939).
Wheat Acts (1932 and 1939). *Byelaws of the Wheat Commission.* Wheat (Examinations and Analyses) Byelaws, 1939. London: The Wheat Commission.
Widdowson, E. M. & McCance, R. A. (1935). *Biochem. J.* **29**, 151.
Woods, C. D. & Merrill, L. H. (1900). *Bull. U.S. Off. Exp. Stas.* no. 85. Cited by Macrae *et al.* (1942).

Hypervitaminosis A

By T. MOORE and Y. L. WANG, *Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*

(*Received* 22 *March* 1945)

Many forms of injury are known to occur in experimental animals when they are dosed with greatly excessive amounts of oils or concentrates containing vitamin A. Takahashi, Nakamiya, Kawakami & Kitasato (1925) tested the effect of excess of their crude concentrate 'Biosterin' when given orally to rats and mice, and observed loss of hair, emaciation and paralysis of the hind legs. After periods varying from a few days to several weeks the animals died, and at autopsy fatty degeneration of the liver,

kidney and heart was found, together with hyperaemia, and sometimes haemorrhage in the intestines and lungs. Both Takahashi *et al.* and later Matsuoka (1934) found that excess of vitamin A concentrate was also toxic when injected, usually causing death with cramp in less than an hour. The principal observations of the Japanese workers in regard to the ill effects of excess of vitamin A concentrates when given orally were confirmed by Harris & Moore (1928), Chevallier, Cornil & Chabré (1934), Simola & Kalaga (1934), Lewis & Reti (1934), Ypsilanti (1935) and Ocana (1935).

Another remarkable lesion, which escaped notice until highly potent concentrates became available, was described by Collazo & Rodriguez (1932) and Bomskov & Seeman (1933). The skeletons of rats which had received excess of concentrate became so fragile that the animals incurred fractures of the large bones of the legs and shoulders in the course of the limited movements possible while in captivity in small cages. Sometimes the broken ends of the bone were ankylosed with the formation of large irregular calluses (Davies & Moore, 1934). Further observations on these skeletal lesions were made by Strauss (1934), Hoff & Jeddeloh (1934), Vedder & Rosenberg (1938) and Wesaw, Wronski, Wroblewski & Wroblewski (1938). Recently, a further injury has been reported by Rodahl & Moore (1943), whose rats often died from severe internal haemorrhage 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. If not a distinct phenomenon it must at least be considered a separate aspect of a general liability to haemorrhage.

While, however, it is well established that concentrated sources of vitamin A are toxic when given in great excess, some doubt has remained as to 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. Undoubtedly fish liver and body oils may be injurious for several distinct reasons unrelated to their content of vitamin A. Agduhr (1926, 1928) noticed the ill effects of cod-liver oil on the musculature of various animals, and pointed out that the oil was still injurious after its vitamin A had been destroyed. Yamamoto (1934) and later Yoshida (1937) concluded that the glyceride fraction, and not vitamin A, was responsible for the adverse action of many marine oils on rats. Hartwell (1927), who found that female rats succumbed to uterine haemorrhage when allowed to become pregnant on diets rich in cod-liver oil, tentatively ascribed this lesion not to vitamin A but to the destructive action of the oil on the vitamin E in the diet. In our experience sperm oil is toxic to rats on account of the presence of cetyl alcohol, which resists absorption; while we have found a stale specimen of sardine oil to be toxic although devoid of vitamin A. It must also be remembered that concentrates rich in vitamin A are often equally rich in vitamin D, which is known to be toxic when given in great excess. Even the original experiments of Takahashi *et al.* (1925) may now be taken as evidence that the toxic effects observed were not all due to vitamin A, since a preparation made from seaweed, and presumably free from preformed vitamin, was no less toxic than their cod-liver oil concentrates.

In crude sources of vitamin A, therefore, many components other than the vitamin might well be responsible for toxicity. Experience with comparatively pure concentrates, moreover, has also suggested to some investigators that the vitamin may not be implicated after all. Although Drigalski (1933) found that concentrates given orally to rats were harmless if the vitamin had been destroyed by ultra-violet irradiation, Matsuoka (1934) observed convulsions in rats after the injection of concentrates which had been freed from vitamin by oxidation or hydrogenation. Conversely a distillate containing the vitamin was not toxic. Hamano (1935), who prepared various crystalline derivatives of vitamin A, found that toxic factors accompanied vitamin A into the unsaponifiable fraction, but could be separated by their inferior solubility in methanol. The purified vitamin did not appear to be toxic. Finally, Vedder & Rosenberg (1938) found no close relationship between vitamin A content and toxicity, as measured by the incidence of bone fractures, in distillates prepared from jew-fish liver oil.

The main purpose of the present experiments, of which a preliminary description has already been given (Moore & Wang, 1943), has been to test the toxicity of vitamin A in its purest available form, i.e. as a crystalline ester. The uterine haemorrhage described by Hartwell (1927) has been reinvestigated to decide whether vitamin A may not be directly involved. The opportunity has also been taken to test the claims of Vedder & Rosenberg (1938) and others that liberal doses of vitamins C and D are beneficial to animals given excess of vitamin A concentrate.

EXPERIMENTAL

The toxicity of pure vitamin A acetate to young rats

Exp. 1. A specimen of crystalline vitamin A acetate ($E^{1\%}_{1\,cm.} = 1500$ for the acetate) was kindly provided by British Drug Houses Ltd. To facilitate administration to rats it was dissolved in arachis oil. Five young male rats weighing 69–79 g. were given a basal diet consisting of casein 200, sugar 500, dried yeast 50, and salt mixture 50 parts. A measured amount of the solution of vitamin A acetate, equivalent to 50,000 i.u. daily during the first days of

the experiment, was given to the animals, after they had fasted overnight, mixed with 3–4 g. of the basal diet. When the dose had been finished the basal diet was given *ad libitum* for the rest of the day. After the first 4 days the dose of vitamin was reduced to 25,000 i.u., since some of the animals would not accept the previous dose.

On the eighth day of the experiment a limping gait, suggestive of tenderness in the legs, was observed in some of the animals. X-ray examination on the tenth day revealed fractures of the limbs in one instance, and the general condition of all the animals was poor. By the twentieth day all the rats had sustained fractures. Care had to be taken to avoid additional fractures during X-ray examination. Exophthalmos was seen in all cases, and in one rat a denudation around the eyes was observed. Post-mortem examination revealed subcutaneous and intramuscular haemorrhages in some of the animals.

X-ray photographs were taken of the rats after excess of vitamin A had been given for 20 days (Pl. 3). It will be seen that the bones become abnormally thin. In one case only there was detachment of the epiphysis from the shaft of the bone, a lesion frequently observed in rather different form in human infantile scurvy. Fractures occurred most often, however, near the centre of large bones. The ends of the bones were sometimes ankylosed with the formation of calluses.

Exp. 2. Young male rats of weight 48–86 g. were given a basal diet similar to that used in Exp. 1, but the dried yeast was increased to 100 parts with a view to improving appetite. Group 1 ('Control') of five animals received 4·5 g. of the basal diet daily mixed with 1·5 g. of plain arachis oil. The vitamin A allowance was 1000 i.u. per week. Group 2 ('Vitamin A excess') received the same diet, but the arachis oil contained 40,000 i.u. of vitamin A acetate daily. Group 3 ('Oxidized vitamin A') had the same diet as group 2, but the oil had been aerated on a boiling water-bath until the vitamin A, as measured by the antimony trichloride reaction, had all been destroyed.

In the group given excess of vitamin A acetate one rat had a limb fractured after 7 days, while within 14 days all the rats in this group had fractures. The animals were miserable in appearance, and gained no weight during the experiment. At autopsy intramuscular haemorrhages were found in some instances. In contrast the rats, both in the control group and in the group given oxidized vitamin A, remained well, had neither fractures nor haemorrhage, and made average gains in weight of 18 and 12 g. respectively during the first 14 days of the experiment.

Mineral content of the bones in hypervitaminosis A. The ash content of the pooled tibiae of the control group, on a fat-free basis, was 52·1 %, while for the group given excess of vitamin A the ash content was 51·3 %. Contrary to expectations no significant difference was therefore detected in hypervitaminosis A.

Ascorbic acid and hypervitaminosis A. In parallel with the preceding groups another five rats were given excess of vitamin A, but with the addition of 10 mg. daily of ascorbic acid. Two animals had fractures on the seventh day of the experiment, and the remaining three had developed fractures, and were in a miserable condition, when they were killed between the ninth and eleventh days. At autopsy intramuscular or subcutaneous haemorrhage was found in all these rats. The ascorbic acid did not therefore alleviate the injurious effects of the excess of vitamin A at the doses in which the two vitamins were given.

Estimations of ascorbic acid by the indophenol method in the livers, adrenals and urine of the preceding groups 1 and 2 also failed to reveal any significant effect of excessive vitamin A on ascorbic acid metabolism:

	Mean wt. of liver (g.)	Ascorbic acid in Liver (mg./g.)	Adrenals (mg./g.)	Urine/rat for 24 hr. (mg.)
Group 1 (control)	6·0	0·11	3·7	0·4
Group 2 (excess of vitamin A)	5·3	0·09	3·3	0·4

Calciferol and hypervitaminosis A. Five more rats received the same diet with excess of vitamin A, and with the addition of 1 mg. daily of calciferol. Fractures developed in all these animals within 11 days, and in some rats haemorrhagic lesions round the eyes were noticed. At autopsy no intramuscular haemorrhage was found, but the muscles of the legs had a purple colour suggestive of venous congestion.

The injection of vitamin A. When young rats were injected with massive doses of vitamin A their reaction never amounted to more than a temporary cramping and twitching of the muscles of the hind legs. In no instance did an animal die as the result of this treatment.

In six rats, which had single doses of 40,000–90,000 i.u. of vitamin A acetate dissolved in arachis oil by either intraperitoneal or subcutaneous injection, no cramping, nor any other injury, was observed. Another animal received an intraperitoneal injection of about 400,000 i.u. of free vitamin A alcohol, prepared from the acetate and dissolved in arachis oil, without developing cramp. An intraperitoneal injection of 25,000 i.u. of vitamin A as halibut-liver oil, however, caused cramping in one rat after 40 min. The condition persisted for over 2 hr., but the rat had recovered by the following

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1. Normal rat.

Fig. 2. Hypervitaminosis A of 20 days' standing. Among the abnormalities are: (i) fractured femurs; (ii) fractured tibia; (iii) ulna and radius are abnormally thin.

Fig. 3. Hypervitaminosis A. Another rat. Fracture of radius and detachment of epiphysis from shaft of bone.

Fig. 4. Hypervitaminosis A. Another rat. Fracture of tibia and fibia. The bones are ankylosed with callus formation.

day. Three other rats after intraperitoneal injections of 50,000 i.u. as halibut-liver oil had no cramp, but an animal injected with 100,000 i.u. had a short attack which started within 5 min. after the injection. Of three rats which had massive injections of a crude commercial concentrate of fish-liver oil containing 25,000 i.u. of vitamin A/g. two developed cramping within about an hour, but recovered. The third appeared miserable for a short time, but had no cramp and recovered rapidly. In all instances in which abnormality was observed it was difficult to decide whether the cramp was genuine, or merely the voluntary effort of the animal to adopt a position to overcome discomfort.

The effect of excessive vitamin A on gestation in the rat

Several series of experiments were carried out to decide whether the uterine haemorrhage observed by Hartwell in pregnant rats given large amounts of cod-liver oil can be ascribed to vitamin A. The usual procedure was to take mature females, which had received a mixed diet of natural foodstuffs, and to place them together with males until spermatozoa were detected in the vaginal smear. The male was then removed, and the diet of the female was changed, immediately or after an interval, to a purified ration which contained vitamin A dissolved in an oily medium. In most of the experiments the diet used contained casein 20 %, sugar 40 %, dried yeast 5 %, salt mixture 5 % and fat 30 %, the solid components and fat being mixed together just before feeding. In some experiments the composition of the diet was varied slightly, the main differences being a reduction in the fat to 20 % and the replacement of the dried yeast by an equal amount of marmite. No evidence was obtained to suggest that these minor alterations significantly affected the course of gestation. On the other hand, the incidence of haemorrhage was vitally affected by the stage at which excess of vitamin A was introduced into the diet.

Massive doses of vitamin A. Vitamin A acetate was given at the rate of 45,000 i.u. daily made up in arachis oil as 20 % of the diet. Other rats received 30,000–40,000 i.u. vitamin A daily as halibut-liver oil. The specimen used was kindly supplied by Allen & Hanburys Ltd., and contained 50,000 i.u./g. It was diluted with arachis oil to make 30 % of the diet. Similar results were obtained with both sources of vitamin A and for the sake of simplicity they may be combined (see table at head of next column). Haemorrhage was first noticed between the fifteenth and twenty-first day of pregnancy in different animals. Inspection at autopsy of the uteri of rats which had died from haemorrhage suggested that the bleeding occurred at the junction of the placenta and endometrium. The placenta was sometimes partially detached, and the endometrium eroded. Histological examination indicated the presence of extensive extravasations of blood at the junction of the placenta and endometrium, but in view of the presence of maternal blood sinuses in this region during normal pregnancy the certain detection of abnormality was difficult. In agreement with the view that the retro-placental haemorrhage had occurred suddenly the structure of the foetuses appeared to be more or less normal.

Vitamin A given	Total no. of rats in group	No. of cases of: Uterine haemorrhage	Failure of implantation or resorption	Litters, dead or alive, without fatal haemorrhage
(*a*) Within 10 days after coitus	13	6	6	1
(*b*) More than 10 days after coitus	12	0	0	12

It must be emphasized that litters have been counted as 'delivered' whether alive or dead. In practice the animals could not be watched continuously, and often it was difficult to decide whether neglected litters had been born alive or dead. 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.

High doses of vitamin A. A veterinary cod-liver oil 'compound', containing 500 i.u. of vitamin A/g., was given as 20 % of the diet from the time of fertilization. The daily intake of vitamin was about 1000 i.u. The results were:

	Total no. of rats	No. of cases of: Uterine haemorrhage	Failure of implantation or resorption	Litters, dead or alive, without fatal haemorrhage
Vitamin A given at coitus	6	1	1	4

In this experiment marmite was given in place of the usual dried yeast. In one of the four rats which survived parturition the latter was accompanied by more profuse haemorrhage than is usual. Of five other rats which were given the oil as 30 % of their diet, four had litters. In the remaining rat no signs of implantation were found at autopsy.

Dosing with marine oils low in vitamin A. A stale specimen of sardine oil, several years old and brown in colour, was given to two rats as 30 % of the diet from the time of coitus. Fresh whale oil was given to six other rats. For the purpose of another in-

vestigation a diet containing 30% of the oil was given not only during pregnancy but for 4 months before mating. Three of these animals had been dosed with tocopherol since first receiving the whale oil, while the other three had not. The results were:

Oil given at coitus	Total no. of rats	No. of cases of: Uterine haemorrhage	Failure of implantation or resorption	Litters, dead or alive, without fatal haemorrhage
Stale sardine oil	2	0	1	1
Whale oil + tocopherol	3	0	1	2
Whale oil − tocopherol	3	0	3	0

Both rats having sardine oil declined in weight throughout the experiment and became very emaciated. The rats having whale oil were not emaciated, and all appeared superficially to be in good health. No abnormal haemorrhage was noticed in any of the rats receiving either oil.

The incidence of uterine haemorrhage in relation to the vitamin A intake. In all these experiments on reproductive abnormalities uterine haemorrhage therefore only resulted when excess of vitamin A was given within 10 days after coitus. The incidence of haemorrhage in rats given oils within this period may be summarized:

Daily vitamin A intake (i.u.)	Incidence of uterine haemorrhage
40,000	6 out of 13
1,000	1 out of 11
0–200	0 out of 8

Haemorrhage in adult non-pregnant rats given excess of vitamin A. In the experiments on the effects of vitamin A acetate on pregnant rats, described in a preceding paragraph, haemorrhage in sites other than the uterus was observed in three instances. In two animals subcutaneous haemorrhages were found in the hind legs. A careful examination failed to reveal any corresponding fracture of the bones. The third rat apparently bled to death as the result of a minor injury caused by self-inflicted scratching behind the ear.

We have also observed haemorrhage in some male rats which for the purpose of another investigation had been given a diet deficient in vitamin E, with the subsequent addition of 36,000 i.u. of vitamin A daily as halibut-liver oil. Only two out of six rats were affected. One died suddenly after receiving the halibut-liver oil for 17 days. There was no premonitory loss in weight, and at autopsy normal intraperitoneal and subcutaneous fat deposits were found. The cause of death was massive haemorrhage in the spleen, the capsule of which was detached and greatly distended with blood. The second rat, which in addition to halibut-liver oil received adequate doses of tocopherol, suffered sudden and complete paralysis of the hind legs after 18 days, again without any loss in weight. Post-mortem examination revealed haemorrhage in and around the bladder, in the pectoral muscles, and inside the cranial cavity at the base of the skull.

DISCUSSION

The lesions in hypervitaminosis A. The results described above indicate that vitamin A in the purest available form is toxic when given in great excess. It is improbable, however, that all the types of lesions which have been reported as the result of overdosing with crude concentrates are equally specific. Skeletal fractures and haemorrhages are, in our experience, the most characteristic lesions in genuine hypervitaminosis A. The fractures occur most consistently in young growing rats, and were found without exception in the present experiments. Haemorrhage is also frequent, if not invariable, in the fractured limbs. In adult rats the bones are probably more resistant to fractures, since we detected no breakages in our older animals. Haemorrhage, however, was frequent, although irregular in its incidence, distribution and severity. Bleeding was sometimes found in the limbs of rats which appeared to be in good health before they were killed. In others heavy internal haemorrhage into the viscera, or external haemorrhage through a minor wound, was rapidly fatal. The uterine haemorrhage in pregnant rats, originally described by Hartwell (1927), appears to be a special manifestation of the general liability to haemorrhage in hypervitaminosis A.

In many of our animals a rawness of the skin was often seen around the eyes, nose and mouth. This injury was undoubtedly due to overdosing with vitamin, but in view of the wide variety of oils which produce skin lesions it is to be considered a less specific lesion than fracture or haemorrhage. We have been unable to reproduce the fatal cramps which Matsuoka (1934) observed as the result of injecting vitamin A concentrates. He was, however, fully aware that the effect could be produced by substances other than vitamin A.

Hypervitaminosis A and scurvy. Mouriquand & Michel (1922) claimed that cod-liver oil antagonized vitamin A in guinea-pigs, causing scurvy even when lemon juice was given. Collett & Eriksen (1938) supported this contention with regard to cod-liver oil, but found that a moderate excess of vitamins A and D had no injurious effect. Although normally the rat is not liable to scurvy, since it is able to synthesize ascorbic acid, Vedder & Rosenberg (1938)

ERRATA

Hypervitaminosis A.
By T. Moore & Y. L. Wang
Volume 39 (1945), No. 3, p. 226, col. 2, line 7 from bottom

for vitamin A *read* vitamin C.

have more recently pointed out that the symptoms which they observed in their animals given excess of fish-liver oil suggested this disease. Thus failure of growth and abnormal fragility of the bones were associated with haemorrhage. In their experiments bleeding occurred particularly at the eyes and nose, but also in mucous membranes in other sites. Our observations of intramuscular and subcutaneous haemorrhage in the limbs brings the picture in hypervitaminosis A even nearer to that seen in scurvy. According to Hess (1920) haemorrhages into the muscles or between the muscle planes are very common in scurvy in adult human subjects, while similar haemorrhages are also frequent in experimental scurvy in the guinea-pig. Massive haemorrhages in the viscera, which often occurred in our adult rats, are less frequent in the human subject, but Eddy & Dalldorf (1941) state that bleeding may take place in any organ, and may vary in size from petechiae to huge extravasations. Their report of a case of fatal haemorrhage into the pericardium in a human subject recalls the observation of the same lesion in a hypervitaminotic rat by Rodahl & Moore (1943).

The finding that young rats are more prone to bone fractures than older animals also recalls the greater incidence of skeletal lesions in infantile than in adult human scurvy. It must be noted, however, that in rats with hypervitaminosis fractures near to the centre of the shaft of the bone are the most prominent feature. The separation of the epiphysis, and fractures in the region near the growing end of the bone to which the human infant is particularly vulnerable, are sometimes present, but perhaps less conspicuous.

A detailed discussion of the points of similarity and difference between the gross lesions in hypervitaminosis A and in scurvy is made difficult by the variations which exist between the three modifications of scurvy as found in juvenile and adult human subjects and in the experimental guinea-pig. Even if we were to make the assumption that the symptoms in hypervitaminosis A are due to a conditioned form of scurvy, it would be too much to expect that the combination of lesions in the rat should either resemble one particular form of scurvy as found in another animal, or should include all the lesions which are found in all forms of scurvy. While, however, the anatomical resemblance between the lesions in hypervitaminosis A and in scurvy is in our opinion largely dependent on our interpretation of what the term 'scurvy' implies, we have been unable to confirm the view that a secondary deficiency of vitamin C can be detected in hypervitaminosis A by biochemical means. Thus we were unable with pure vitamin A acetate either to repeat the observation of Vedder & Rosenberg (1938) that ascorbic acid affords protection against excess of vitamin A concentrate, or to detect any abnormality in ascorbic acid metabolism in our hypervitaminotic rats.

Hypervitaminosis A and vitamins D and B. Thoenes (1935) has asserted that vitamins 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. The reverse finding of Vedder & Rosenberg (1938) that vitamin D (50,000 i.u. daily) gives protection against excess of vitamin A concentrate is therefore not surprising. In our experiments, however, no benefit was derived from doses of 1 mg. of calciferol. In view of the toxicity of great excess of calciferol it may be necessary to adjust the dose carefully in order to provide protection against excess of vitamin A, and our findings must be taken to indicate only that no protection was exerted at the levels chosen. Additional allowances of the vitamin B complex have also been found to be beneficial to animals given excess of marine oils (Harris & Moore, 1928; Bell, Gregory & Drummond, 1933). According to Yamamoto (1934) and Yoshida (1937), however, the extra vitamin B complex, of which the active constituent is riboflavin, counteracts the injurious effect of higher unsaturated acids rather than of vitamin A. In the present experiment the effect of varying the allowance of the vitamin B complex was not investigated systematically, but such changes as were made from time to time had little or no obvious effect on the development of hypervitaminosis A.

SUMMARY

1. Hypervitaminosis A was induced in rats by giving them orally massive doses of either crystalline vitamin A acetate or halibut-liver oil.

2. The most characteristic lesions were skeletal fractures, which occurred most consistently in young animals, and internal or external haemorrhage. These injuries bore some superficial resemblance to those found in human and experimental scurvy.

3. The uterine haemorrhage in pregnant rats given excess of cod-liver oil, first reported by Hartwell, was reproduced in animals given excess of cod-liver oil, halibut-liver oil or vitamin A acetate. This haemorrhage appears to be a special manifestation of the general liability to haemorrhage in hypervitaminosis A.

Our thanks are due to Dr L. J. Harris for his valuable criticism, to British Drug Houses Ltd. for a generous gift of vitamin A acetate, to Prof. W. J. Hamilton of St Bartholomew's Hospital for histological examinations, to Miss A. C. Cooper and Miss J. E. Payne for the care of the experimental animals and to Mr A. W. Davies for X-ray photography. Y. L. Wang is indebted to the Medical Research Council for a personal grant.

Note added in proof 30 *July* 1945. Since this paper was written, Light, R. F., Alscher, R. P. & Frey, C. N. (1944, *Science*, **100**, 225) have reported that hypoprothrombinaemia, which often causes cerebral haemorrhage, may be induced in rats by massive doses of vitamin A, and may be prevented by the simultaneous addition of vitamin K.

REFERENCES

Agduhr, E. (1926). *Acta paediatr., Stockh.*, **6**, 165.
Agduhr, E. (1928). *Acta paediatr., Stockh.*, **7**, 289.
Bell, M. E., Gregory, E. & Drummond, J. C. (1933). *Z. Vitaminforsch.* **2**, 161.
Bomskov, C. & Seeman, G. (1933). *Z. ges. exp. Med.* **89**, 771.
Chevallier, A., Cornil, L. & Chabré, P. (1934). *C.R. Soc. Biol., Paris*, **115**, 541.
Collazo, J. A. & Rodriguez, J. S. (1932). *Klin. Wschr.* **12**, 1732, 1768.
Collett, E. & Eriksen, B. (1938). *Biochem. J.* **32**, 2299.
Davies, A. W. & Moore, T. (1934). *Biochem. J.* **28**, 288.
Drigalski, W. (1933). *Klin. Wschr.* **12**, 308.
Eddy, W. H. & Dalldorf, G. (1941). *The Avitaminoses*, 2nd ed., p. 327. London: Baillière, Tindall and Cox.
Gross-Selbeck, C. (1935). *Klin. Wschr.* **14**, 61.
Hamano, S. (1935). *J. agric. chem. Soc. Japan*, **11**, 341.
Harris, L. J. & Moore, T. (1928). *Biochem. J.* **22**, 1461.
Hartwell, G. A. (1927). *Biochem. J.* **21**, 1076.
Hess, A. F. (1920). *Scurvy Past and Present.* Philadelphia and London: J. B. Lippincott Co.
Hoff, F. & Jeddeloh, B. (1934). *Z. ges. exp. Med.* **95**, 67.
Lewis, J. T. & Reti, L. (1934). *Rev. Soc. argent. Biol.* **10**, 302.
Matsuoka, M. (1934). *Bull. Inst. phys. chem. Res. Japan*, **13**, 1267.
Moore, T. & Wang, Y. L. (1943). *Biochem. J.* **37**, *Proc.* viii.
Mouriquand, G. & Michel, P. (1922). *C.R. Soc. Biol., Paris*, **1**, 1170.
Ocana, T. (1935). *Rev. Soc. argent. Biol.* **11**, 147.
Rodahl, K. & Moore, T. (1943). *Biochem. J.* **37**, 166.
Simola, P. E. & Kalaga, T. (1934). *Skand. Arch. Physiol.* **71**, 31.
Strauss, K. (1934). *Beitr. path. Anat.* **94**, 345.
Takahashi, K., Nakamiya, Z., Kawakami, K. & Kitasato, T. (1925). *Sci. Pap. Inst. phys. chem. Res., Tokyo*, **3**, 81.
Thoenes, F. (1935). *Dtsch. med. Wschr.* **61**, 2079.
Vedder, E. B. & Rosenberg, C. (1938). *J. Nutrit.* **16**, 57.
Wesaw, W., Wronski, B., Wroblewski, A. & Wroblewski, B. (1938). *Klin. Wschr.* **17**, 777.
Yamamoto, I. (1934). *Bull. Inst. phys. chem. Res. Japan*, **13**, 1.
Yoshida, M. (1937). *J. agric. chem. Soc. Japan*, **13**, 120.
Ypsilanti, P. H. (1935). *Klin. Wschr.* **14**, 90.

Influence of Halides on the Oxidation of Ascorbic Acid

2. ACTION OF Cl^- ON THE CUPRIC-CUPROUS SYSTEM

By L. W. MAPSON, *The Nutritional Laboratory, University of Cambridge, and the Medical Research Council*

(*Received* 23 *February* 1945)

Several workers have shown that Cl^- retards the aerobic oxidation of ascorbic acid by Cu (De Caro & Giani, 1934; Kellie & Zilva, 1935; Mystkowski & Lasocka, 1939). Mapson (1941) found that Br^- and I^- inhibited this reaction even more than does Cl^-, but that F^- had no inhibitory effect. Moreover, at low concentrations, the ions Cl^-, Br^- and I^- increased the catalytic activity of the Cu in the reaction, the degree of acceleration being influenced *inter alia* by pH, concentration of Cu and oxygen tension. An attempt has been made in the present work to determine the mechanism by which the halide ions act on this system.

EXPERIMENTAL

Methods

Reagents. The chemical salts used were of A.R. purity. Glass-distilled water was used.

Peroxidase. Szent-Györgyi (1928), Tauber (1936) and Huzak (1937) found that only crude preparations of peroxidase were able to promote the oxidation of ascorbic acid by H_2O_2, whereas purified preparations were unable to do so. This we were able to confirm. Dr T. Mann kindly set at our disposal a series of peroxidase preparations ranging from crude extracts to material of high purity; the purest preparations had no catalytic effect on the oxidation of ascorbic acid by H_2O_2, though they did after the addition