HYPERVITAMINOSIS A IN THE CHICK

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Bone lesions have been described in animals given large doses of vitamin A. The bones are reduced in thickness and fractures are frequent (Moore, 1957). Much of this work has been done on the rat (Moore and Wang, 1945; Wolbach, 1947; Thompson and Pitt, 1960), but lesions have been described in other species including guinea-pigs (Wolbach, 1947), calves (Grey, Nielsen, Rousseau, Calhoun and Eaton, 1965) and man (Persson, Tunell and Ekengren, 1965). Rigdon, Rude and Bieri (1951) did not find lesions in the bones of hypervitaminotic ducklings but in contrast Wolbach and Hegsted found that hypervitaminosis A produced bone lesions in chicks (1952c) and in ducks (1953). Poumeau-Delille (1943) and Walker, Eylenberg and Moore (1947) recorded a marked reduction in the number of red blood cells in hypervitaminotic rats.

The purpose of the work reported here was to investigate some aspects of the gross, histo- and chemical pathology of hypervitaminosis A in chicks and to contrast the changes in bone with those observed in vitamin A deficient birds by Howell and Thompson (1967a, b).

MATERIALS AND METHODS

Thirty 1-day-old male chicks of Thornber’s 404 strain were fed a commercial diet for the duration of the experiment. From the time that they were 7 days old the birds were weighed daily and 15 of them received a dose of 20 mg. retinyl acetate per 100 g. body weight per day. This was deposited into the oesophagus using a polythene tube. The other 15 birds were used as controls.

At 14, 21 and 28 days of age, 3, 4 and 5 birds respectively were killed from each of the 2 groups. They were anaesthetised with chloroform, given an intracardiac injection of heparin and exsanguinated, the blood being collected. The carcase was immediately perfused via the heart with normal saline, the right leg was removed and stored at -20° and the remainder of the carcase perfused with formol acetate. After storage in formalin, material was taken for histological examination from the cerebrum, cerebellum, pons and medulla, a transverse section of the face at the level of the posterior commissure of the external nares, a longitudinal section of the bones of the base of the skull, a transverse section of the first lumbar vertebra, a transverse section of the midshaft of the right ulna and a longitudinal section of the lower third of the left tibiotarsus. Tissues were embedded in paraffin wax, sections were cut at 5 μ and stained with haematoxylin and eosin. Sections of the tibiotarsus were also stained with toluidene blue.

The blood samples were examined for packed cell volume, number of red blood cells and haemoglobin content measured as oxyhaemoglobin in a spectrophotometer. The acid and alkaline phosphatases in plasma were estimated by the method of King and Armstrong (1934).

Approximately 2 months after collection the right legs were removed from the -20° refrigerator, and all extraneous tissue was trimmed off the tibiotarsus. These were then weighed and the distances from the top of the tibial crest to the base of the distal inter-

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condylar notch, and the midshaft circumferences, were measured. The periosteum was removed for 2-5 mm. on each side of the midpoint of the shaft of each bone and weighed. Samples of cortical bone weighing 20–50 mg. were obtained from the full thickness of the bone at this site and samples weighing 50–80 mg. were removed from the distal extremity of the bone extending from the articular cartilage to the epiphyseal plate and for an equivalent distance into the metaphysis. These tissues were prepared by the method of Jeffree (1959) and their acid and alkaline phosphatase content was estimated by the method of King and Armstrong (1954).

RESULTS

Gross Pathology.—On the seventh day of the experiment birds given retinyl acetate were seen to have inflamed skin around the external nares, commissures of the beak and eyes. Exudate and food material was encrusted over these areas. The hypervitaminosis birds did not at first grow at the same rate as the controls and 2 of them died at the end of the first week of the experiment. After 2 weeks, however, the birds remaining in this group seemed to be less affected by the excessive amounts of vitamin A and their body weights approached those of the controls.

The weights, lengths, midshaft circumferences and periosteal thicknesses of the tibiotarsi are given in Table I. They show that, when compared with the controls, the hypervitaminotic birds had shorter, narrower, lighter tibiotarsi with thinner periostea. The only observation that does not fit this pattern is the midshaft circumference of the 14-day-old birds.

Histopathology.—Similar lesions were observed at all sites and at all ages in the bones of the hypervitaminotic birds. The periosteum was thinner and had a smoother surface than that of the controls; there was an increase in the fibrous tissue component and a marked decrease in the number of osteoblasts. The osteoblasts that were present appeared to be less active (Figs. 1 and 2). Similarly,

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<th>Table I.—Mean Tibiotarsis Weights, Lengths, Shaft Circumferences, and Periosteal Thickness, Expressed as sq. cm. Periosteum/g.</th>
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<tr>
<td>Age (days)</td>
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<td>Group No./group</td>
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<tr>
<td>Mean weight (g.)</td>
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<td>Mean length (mm.)</td>
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<td>Mean circumference (mm.)</td>
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<td>Squ. cm. periosteum/g.</td>
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EXPLANATION OF PLATES.

Fig. 1.—Longitudinal section of the cortical bone of the tibiotarsus of a bird that had been given retinyl acetate for 1 week. The subperiosteal osteoblasts appear inactive and are few in number. H. and E. ×42.

Fig. 2.—Longitudinal section of the cortical bone of the tibiotarsus of a control bird of the same age as that in Fig. 1. Numerous and active subperiosteal osteoblasts are present. H. and E. ×42.

Fig. 3.—Section through the epiphyseal cartilage of the tibiotarsus of a bird that had been given retinyl acetate for 1 week. Vascular channels are infrequent, and there is much cartilage. H. and E. ×6.

Fig. 4.—Section through the epiphyseal cartilage of the tibiotarsus of a control bird of the same age as that in Fig. 3. The vascular channels and the cartilage are normal. H. and E. ×6.
Baker, Howell and Thompson.
in the subperiosteal bone the osteoblasts were fewer and less active than in the controls. Evidence of an effect on osteoclasts was not found.

The sites of endochondral ossification in the birds given retinyl acetate all showed similar changes, and the degree of change appeared to be proportional to the rate of growth at that particular site. There was a marked increase in the thickness of the zone that Woblach and Hegsted (1952a) called the epiphyseal or growth cartilage, and this increase was due to larger numbers of apparently healthy hyperplastic chondrocytes and their matrix. Associated with this lesion was a marked decrease in the number and a slight increase in the size of the invading vascular channels (Figs. 3 and 4). In sections of the lower extremity of the tibiotarsi stained with toluidene blue there appeared to be an equal degree of metachromasia in control chicks and in those that had received retinyl acetate. The number of nests of uncalcified hypertrophic chondrocytes in the trabeculae of the primary spongiosa was increased in the hypervitaminotic birds. The cortical bone was thinner and denser than that of the controls, and had a large number of cement lines.

Two of the 4 hypervitaminotic birds killed when 21 days of age had shrunken cells with pyknotic nuclei in the granular layer of cerebellar folia. Evidence of ventricular dilatation and brain swelling was seen in 3 of the 5 hypervitaminotic birds killed when 28 days old.

The skin and subcutis in the region of the external nares of the hypervitaminotic chicks showed a low grade inflammatory change with degeneration and necrosis of the superficial epithelium. Significant changes were not seen in the nasal mucosa nor were lesions observed in the non-ossifying cartilage of the nasal turbinates.

Chemical Pathology.—The results of the analysis of the blood and bones are given in tables II and III. The blood and bone alkaline phosphatase levels were

| Table II.—Plasma Acid and Alkaline Phosphatase of Normal and Hypervitaminotic A Chicks Expressed as King Armstrong Units/100 ml. Plasma |
|---|---|---|---|---|
| Age (days) | 14 | 21 | 28 |
| Group No./group | Hyper Normal | Hyper Normal | Hyper Normal |
| Alkaline phosphatase | 2 3 | 4 4 | 5 5 |
| Acid phosphatase | * | * | * |

* Blood samples too small for examination.

lower in the hypervitaminotic birds than in the controls except in the first group of birds killed after retinyl acetate had been given for 7 days. At this age the control and hypervitaminotic birds had similar levels in cortical bone. This agrees with the results of shaft circumference measurements taken at this age (Table I).

The acid phosphatase levels were moderately elevated in the blood, epiphyseal and cortical bone of hypervitaminotic birds killed when 21 or 28 days of age, and in the periosteal tissue of such birds killed when 28 days old (Table III).

Haematology.—The results of the red blood cell counts, packed cell volumes and
Our investigation of the chronic toxicity of vitamin A indicates that its effects on bone structure are much less marked in the chick than in the rat, in which bone resorption results in multiple fractures of the long bones. We have been unable to demonstrate marked resorption in the long bones of chicks even when large quantities of retinoic acid have been administered (Howell and Thompson, unpublished), a procedure which results in extremely severe bone fragility in rats (Thompson and Pitt, 1960).

Our findings support those of Wolbach and Hegsted (1952c) in that the administration of large doses of vitamin A does produce bone lesions in the chick but we cannot agree with these authors concerning the detail of these changes. The bones were shorter, narrower and lighter than those of the controls. Histologically there were 2 main abnormalities in the structure of the bones of hypervitaminotic birds; there appeared to be an inhibition of osteoblasts (Fig. 1) and there was an increased amount of epiphyseal cartilage present (Fig. 3).

The more fundamental lesion appeared to be an inhibition of osteoblasts, few of these cells were seen and those present were inactive. Blood and bone alkaline phosphatase levels were lower in the hypervitaminotic birds than in the controls and this was thought to confirm the histological finding of a low osteoblastic activity. It would be interesting to see if complete inhibition of osteoblasts would be produced by the administration of a higher level of retinyl acetate than that given in these experiments. Wolbach and Hegsted (1952c) did not comment directly upon the action of hypervitaminosis A on osteoblasts but they recorded...
that "the deep layer of the periosteum is less cellular than that of normal controls", and they recorded that the cortical bone was very dense and that compact bone seemed to replace cancellous bone more rapidly than in the controls.

Irving (1949) had concluded that the inhibition of osteoblastic activity was the important action of high levels of vitamin A in the rat. He found that osteoclasts were not directly affected and this would appear to be so in the chick. The importance of vitamin A in the control of osteoblastic activity has been reported by Mellanby (1944; 1950) and Irving (1949) for mammals and by Howell and Thompson (1967a, b), for birds. The results of the investigation reported here on hypervitaminosis A and of the experiments on vitamin A deficiency (Howell and Thompson, 1967a, b) lead us to the conclusion that in the chick as in the rat (Irving, 1949) vitamin A controls the activity of osteoblasts. While we would not subscribe to the view that the mechanisms underlying the abnormalities resulting from vitamin A deficiency and vitamin A excess are necessarily or obviously related to one another, it is true that vitamin A deficiency results in stimulation of the activity of osteoblasts and hypervitaminosis results in its suppression.

The failure to remove epiphyseal cartilage at the normal rate is at variance, with the findings of Wolbach and Hegsted (1952c), who described a narrowing of this cartilage zone. Wolbach and Hegsted (1952b) and Howell and Thompson (1967a) found an increase in the thickness of epiphyseal cartilage and a reduction in the number of vascular tunnels in the long bones of vitamin A deficient chicks, and Carlton and Henderson (1964) had recorded similar changes in copper deficient chicks. Howell and Thompson (1967a) suggested that this lesion was associated with inanition, for the change appeared some days after the vitamin A deficient chicks had ceased to grow at the normal rate, and was also associated with a fibroblastic replacement of the haemopoietic tissue of the bone marrow. The results reported here would not contradict this hypothesis, although bone marrow fibrosis was not seen.

The cell damage and swelling seen in the central nervous system of some of the hypervitaminotic birds was thought to have been due to mechanical factors associated with the failure of bone formation. The skin lesions were restricted to the commissures of the beak, nose and eyes of the hypervitaminotic chicks. The histological appearance of the lesion was of a non-specific inflammatory response with degeneration and necrosis of the epithelium. In previous experiments (Howell and Thompson, unpublished) the vitamin A had been given by pipette into the mouth and some of this had run over the beak. In these birds the skin lesions were more severe and were also found on the feet. In the experiments reported here the vitamin A acetate was deposited into the oesophagus and only a small amount was ever present in the mouth. This reduced the severity and extent of the skin lesions, and we presume that they are a local response to irritation. The haematological findings do not support those of Poumeau-Delille (1943) and Walker, Eylenberg and Moore (1947) in the rat. The hypervitaminotic chicks in our experiments did not become anaemic.

**SUMMARY**

Hypervitaminosis A was induced in 15 chicks by giving daily 20 mg. of retinyl acetate/100 g. body weight. This was first given when the chicks were 7 days old and hypervitaminotic birds were killed together with an equivalent number of
controls when they were 14, 21 and 28 days old. All hypervitaminotic birds grew more slowly and had shorter, narrower, lighter bones than the controls. There was a pronounced effect on osteoblasts and their activity was severely inhibited. This was confirmed by low alkaline phosphatase levels in blood and bone. The significance of the changes in osteoblasts is discussed. The epiphyseal cartilage was abnormally thick and the vascular channels were fewer and wider than in the controls. This was thought to be due to inanition. Inflammatory lesions were present on the skin of the face and these were thought to have been a local response to irritation. The birds were not anaemic.

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