PREVENTION BY CORTISONE OF THE CHANGES IN CARTILAGE INDUCED BY AN EXCESS OF VITAMIN A IN RABBITS

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The administration of large doses of vitamin A to rabbits was previously reported to cause collapse of the ears and loss of chondroitin sulfate from cartilage.[1] The effects were strikingly similar to those demonstrated by Fell and Mellanby[2] when excess vitamin A was added to the culture of embryonic bone explants. In view of the similarities between this property of vitamin A and the action of intravenous papain in the rabbit,[3] and of papain added to embryonic bone cultures,[4] it was suggested that vitamin A might act by causing the release of proteolytic enzymes from cartilage cells or their organelles into the matrix of cartilage. Subsequently, Lucy, Dingle and Fell[5] found that embryonic bone explants contained a protease which was activated by acidification; loss of chrondroitin sulfate from cartilage was demonstrated *in vitro* under conditions in which this enzyme was activated. These investigators then showed that vitamin A caused release of a similar protease, presumed to be a cathepsin, from embryonic cartilage. Postulating that the source of the enzyme might be the lysosomes of cartilage cells, Dingle investigated the action of vitamin A on the lysosomes of rat liver.[6] It was found that vitamin A caused the release of acid protease from suspensions containing the bulk of hepatic lysosomes.

Fell and Thomas found that hydrocortisone inhibited the action of vitamin A on the cartilage of embryonic bone explants.[7] It was then shown that the lysosome-rich fraction taken from the livers of rats pretreated with hydrocortisone released less acid protease than controls upon ultraviolet irradiation.[8] De Duve, Wattiaux and Wibo had previously demonstrated that hydrocortisone diminished the release of acid phosphatase, another lysosomal enzyme, from rat liver granules *in vitro*.[9] On the basis of these observations it was suggested that hydrocortisone had the property of stabilizing lysosomes against several forms of injury, including excess vitamin A. However, two reports contradictory to this

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hypothesis exist, both involving *in vivo* systems. Selye[10] reported that the bone damage produced by hypervitaminosis A in young rats was aggravated by cortisone, and Weissman described synergistic damage in toad tadpoles by simultaneous treatment with hydrocortisone and vitamin A.[11]

The present report concerns the effect of cortisone on hypervitaminosis A in the rabbit. It will be shown that cortisone exerts a definite protective action against an excess of vitamin A palmitate and vitamin A acid in this animal.

Material and Methods

Young albino rabbits of either sex, weighing between 800 and 1,200 gm., were used.

Vitamin A palmitate in a solution of cottonseed oil was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Vitamin A acid was obtained in crystalline form through the courtesy of Hoffman-LaRoche, Nutley, New Jersey. It was suspended in corn oil (100 mg. per ml.) before administration. The vitamin A preparations were administered by gastric intubation.

Cortisone acetate in aqueous suspension (25 mg. per ml.), from Merck and Company, West Point, Pennsylvania, or Philadelphia Ampoule Laboratories, was administered intramuscularly. Hydrocortisone sodium succinate (Solu-Cortef®, Upjohn Company, Kalamazoo, Michigan) was diluted with saline to a final concentration of 1.5 mg. per ml. before intra-articular injection.

The rabbits were killed by an injection of Nembutal ®. Tissues were fixed in 10 per cent buffered formalin. Bones were decalcified in dilute formic acid. Sections were prepared from the lower end of the femur, trachea and ear and were stained with hematoxylin and eosin and with toluidine blue according to the method of Kramer and Windrum.[12]

Acute hypervitaminosis A in the rabbit affects the cartilage of joint surfaces and epiphyseal plates more regularly and severely than that of the ear or trachea.[1] For this reason estimation of the degree of depletion of cartilage matrix was estimated in sections of the distal end of the femur and graded as follows: −, normal metachromatic and basophilic staining of articular and epiphyseal cartilage; ±, equivocal reduction in metachromatic and basophilic staining; +, mild reduction in metachromatic and basophilic staining with little or no thinning of articular cartilage or epiphyseal plates; ++, moderate loss of metachromatic and basophilic staining with slight thinning of articular cartilage and mild to moderate thinning of epiphyseal plates; +++, virtually complete absence of metachromatic and basophilic staining with moderate thinning of articular cartilage and marked thinning of epiphyseal plates.

Experimental Results

Prevention by Cortisone of Cartilage Depletion Following Vitamin A Palmitate

Two experiments were performed in which rabbits given vitamin A palmitate were treated with cortisone. In the first experiment 4 groups of rabbits were used as follows: In group I, 9 rabbits were given 500,000 I.U. vitamin A palmitate every day for 6 days. In group II, 9 rabbits were given vitamin A as in group I. On the day preceding and throughout the period of vitamin A administration, each rabbit also received a sin-

gle daily injection of 12.5 mg. of cortisone. In group III, 9 rabbits received cortisone as in group II. In group IV, 9 control rabbits received no treatment. All the rabbits were killed on the day following the last treatment.

The results of this experiment are summarized in Table I. The rab-

TABLE I

PREVENTION BY CORTISONE OF CARTILAGE MATRIX DEPLETION IN RABBITS TREATED WITH VITAMIN A PALMITATE

Exper. no.	Treatment	No. of rabbits	No. with ear collapse	No. of rabbits with cartilage matrix depletion				
				o	±	+	++	+++ *
1	Vitamin A †	8	4				1	7
	Vitamin A plus cortisone ‡	9	0	2	5	2		
	Cortisone	9	0	9				
	Controls	9	0	9				
2	Vitamin A §	8	4				3	5
	Vitamin A plus cortisone ¶	8	2	3	2	2	1	

* See text for manner of grading.
† Vitamin A palmitate, 0.5 million units daily for 5 days.
‡ Cortisone, 12.5 mg. daily for 6 days.
§ Vitamin A palmitate, 1 million units daily for 3 days.
¶ Cortisone, 25 mg. then 12.5 mg. daily for 3 days.

bits given vitamin A only (group I) showed changes similar to those previously reported.[1] Grossly, in 4 of the 9 rabbits there was collapse of the distal part of the ear. Loss of hair was obvious in all. One rabbit died on the fifth day. Histologically, all the rabbits were found to have moderate or severe depletion of the matrix in articular and epiphyseal cartilage (Figs. 1 and 3). Sections of trachea showed mild focal depletion of cartilage matrix in 5 rabbits. Examination of ear cartilage showed mild reduction of staining in 2 instances.

In the rabbits treated with cortisone during the period of vitamin A administration (group II), the findings were quite different from those just described. None of the rabbits developed ear collapse or showed loss of hair. Two rabbits exhibited mild depletion of articular and epiphyseal cartilage matrix; in 5 rabbits there was only equivocal depletion and in 2 rabbits no alterations were found (Figs. 2 and 4). In sections of ear and trachea no loss of cartilage matrix was evident.

In the rabbits treated with cortisone only (group III) no reduction of metachromatic or basophilic staining of cartilage matrix was encountered. However, as in earlier studies,[13] slight thinning of epiphyseal plates and reduction in the number of newly formed metaphyseal bony trabeculae was observed. This was also observed in rabbits in group II.

In the untreated rabbits (group IV) no abnormalities of cartilage were found.

In the second experiment, using vitamin A palmitate and cortisone, 2 groups of rabbits were employed. Group I consisted of 8 rabbits given 1 million units of vitamin A daily for 3 days. Group II consisted of 8 rabbits given vitamin A as in group I and in addition 25 mg. of cortisone on the day preceding vitamin A treatment and then 12.5 mg. of cortisone daily for 3 days. All rabbits were killed on the day following the last day of treatment.

Cortisone treatment partially prevented the changes due to vitamin A (Table I). Four of the rabbits given only vitamin A developed collapse of the distal portion of the ears. Two rabbits treated with cortisone and vitamin A showed slight curling at the tips of the ears. All of the rabbits given only vitamin A showed severe loss of hair, whereas among the rabbits given cortisone as well, only 3 showed hair loss. Of the rabbits treated with vitamin A, 5 exhibited severe depletion (3+) of articular and epiphyseal cartilage and 3 showed moderate depletion (2+). In the rabbits treated with both cortisone and vitamin A, 3 were found to have normal cartilage, 2 showed equivocal depletion, 2 showed mild depletion (+) and only 1 showed moderate depletion (2+).

Depletion of Cartilage Matrix by Vitamin A Acid and Prevention by Cortisone

Four groups of rabbits were used in these experiments: In group I, 9 rabbits received 250 mg. of vitamin A acid in corn oil (2.5 ml.) daily for 5 days. In group II, 9 rabbits received vitamin A acid as in group I and 50 mg. of cortisone a day for 5 days. In group III, 9 rabbits were given 50 mg. of cortisone daily for 5 days and 2.5 ml. of corn oil daily. In group IV, 9 control rabbits received 2.5 ml. of corn oil by intubation daily for 5 days. All rabbits were killed 4 hours after the last treatment (Table II). Within 24 to 48 hours, 4 animals in group I showed curling

TABLE II

PREVENTION BY CORTISONE OF CARTILAGE MATRIX DEPLETION IN RABBITS TREATED WITH VITAMIN A ACID

Treatment	No. of rabbits	No. with ear collapse	No. of rabbits with cartilage matrix depletion				
			o	±	+	++	+++
Vitamin A *	9	8			3	3	3
Vitamin A and cortisone †	6	o	2	2	2		
Cortisone	9	o	9				
Controls	9	o	9				

* Vitamin A acid, 250 mg. daily for 5 days.

† Cortisone, 50 mg. daily for 5 days.

of the distal third of the ears. By 72 hours 8 of the 9 exhibited this change. In contrast, only 1 animal in group II had curling of the ears; this developed at 72 hours. All of the animals in group I showed a remarkable loss of hair, which began to be quite noticeable at 48 hours. No significant hair loss was apparent in group II. None of the animals in group III or IV had ear collapse or hair loss. In all of the rabbits in group I there was moderate or severe depletion of articular and epiphyseal cartilage matrix (Fig. 5), whereas among animals in group II only 2 rabbits showed definite depletion, which was of mild degree (Fig. 6).

The Local Prevention by Hydrocortisone of Depletion of Cartilage Matrix Induced by Vitamin A

In order to see if the prevention by cortisone of cartilage depletion due to vitamin A was the result of a direct action of the steroid on cartilage, the following experiment was performed.

Ten rabbits were given 750,000 units of vitamin A palmitate by gastric intubation daily for 3 days. During the same period each rabbit received injections of hydrocortisone, 0.15 mg. in 0.1 ml., in the right knee joint and 0.1 ml. of saline into the left knee twice daily. All rabbits were killed one day after the last treatment (Table III). In 3 of the rabbits

TABLE III

EFFECT OF LOCAL HYDROCORTISONE ON ARTICULAR CARTILAGE DEPLETION IN VITAMIN A-TREATED RABBITS *

Knee joint injected with:	Degree of depletion			
	o	±	+	++
Hydrocortisone †	o	7	3	o
Saline	o	4	3	3

* Vitamin A, 0.75 million units P.O. for 3 days.
† Hydrocortisone, 0.15 mg. intra-articularly B.I.D. for 3 days.

the degree of matrix depletion in the articular cartilage was less in the hydrocortisone-injected joint than in the joint injected with saline (Figs. 7 and 8). In 4 rabbits only equivocal depletion was found in both sides, and in 3 rabbits equal, mild depletion of cartilage matrix was present in both joints.

DISCUSSION

The present observation has shown that the depletion of cartilage matrix produced *in vivo* in rabbits by an excess of vitamin A palmitate or acid could be largely prevented by the concurrent administration of cortisone. The degree of protection appeared to be related to the dose of vitamin A employed. At essentially the same dosage of cortisone there was less protection when 1 million units of vitamin A palmitate were

given daily than when 500,000 units were given. The protective action of adrenal corticoids seemed to be the result of a direct effect on cartilage; in rabbits given an excess of vitamin A palmitate, the injection of hydrocortisone into one knee joint partially prevented the depletion of that articular cartilage matrix as compared with the contralateral joint. Aside from the protection against the changes in cartilage, cortisone largely prevented the striking loss of hair seen in rabbits treated with excess vitamin A. The mechanism leading to hair loss is not known.

Vitamin A acid has not previously been shown to cause depletion of cartilage matrix *in vivo,* although such an effect on embryonic limb bone rudiments *in vitro* has been reported by Fell, Dingle and Webb.[14] Thompson and Pitt,[15] in a preliminary report, have shown that vitamin A acid in excess caused lesions in the bones of rats comparable to those produced by other forms of the vitamin.

The present observations provide further support for the hypothesis that the lysosomes of cartilage are rendered more resistant to the effects of hypervitaminosis A by adrenal corticoids. Evidence for the protection of liver lysosomes against the action of an excess of vitamin A *in vivo* and *in vitro* will be presented in a separate paper.[16] Cortisone and its analogues appear to decrease the release of lysosomal hydrolases from liver granules caused by a variety of agents such as bacterial endotoxin,[17] ultraviolet irradiation[8] and exposure to acid pH.[9] DOCA is ineffective in such circumstances.[17]

It is of interest to note that Wolf[18] reported that vitamin A appears to be necessary for the biosynthesis of sulfated mucopolysaccharides in intestine; this synthetic activity seems to reside in a granular fraction whose sedimentation properties are similar to the particles studied by Dingle.[6] It is not clear whether this reflects another action of vitamin A on lysosomes or whether the vitamin in this situation is acting on different cellular elements.

The present findings are in contrast to those reported by Selye[10] and by Weissmann.[11] Selye reported that the simultaneous administration of cortisol and vitamin A to rats resulted in intense bone absorption not observed with vitamin A alone. These experiments differed considerably from ours in that a different species was employed, a different end point looked for (bone resorption instead of cartilage depletion), and the course of treatment was more prolonged. In experiments with larvae of *Xenopus laevis,* hydrocortisone led to acceleration of connective tissue damage and gastrointestinal changes induced by vitamin A alcohol. In recent experiments, however, it was found that the simultaneous administration of hydrocortisone protected larvae against the similar effects produced by vitamin A acid.[19]

If it is assumed that the mechanism of action of excess vitamin A in the experiments of Weissmann[11] and of Selye[10] was the same as in the rabbit, how can these discordant results be explained? It was previously proposed that the acceleration of connective tissue changes observed in larvae by hydrocortisone was due to the release of vitamin A from hepatic stores.[11] The release of stored vitamin A under the influence of cortisone and corticotropin has been demonstrated in the rat by McGillivray[20] and in human subjects by Wang and associates.[21] Since vitamin A acid is not stored to any extent in the liver, the protection by hydrocortisone against vitamin A acid but not against vitamin A alcohol (which is stored in an esterified form in the liver) is consistent with this suggestion. The release of hepatic stores of vitamin A does not appear to be relevant to the experiments reported in this paper, since it was shown by McGillivray[20] that blood levels of vitamin A alcohol (the active form) did not rise significantly until the seventh day after steroid therapy. In our rabbit experiments, cortisone was given for only 6 days. In the experiments of Selye[10] and of Weissmann,[11] on the other hand, prolonged steroid therapy was used and may have resulted in significant elevation of blood levels of vitamin A.

Another, and perhaps more probable, explanation can be offered for the additive effects of hydrocortisone and vitamin A *in vivo*. It has been shown previously that cortisone or hydrocortisone prevent the reconstitution of cartilage matrix following depletion by papain, apparently by a direct inhibitory effect on the synthesis of constituents of the matrix.[3,13] If the circumstances of the experiment are such that the lytic effect of vitamin A is not completely prevented by cortisone, and if cortisone and vitamin A are administered over a relatively long period, there might occur both depletion and suppression of reconstitution of cartilage matrix.

In any case, the observations reported here show that cortisone has the capacity of blocking the effects of excess vitamin A *in vivo* under appropriate circumstances. It is of interest that the combined effects of cortisone and vitamin A in the rabbit are matched by those observed in an *in vitro* system[8] and in tissue culture.[7] In view of the demonstration by de Duve and co-workers[9] that the stability of lysosomes *in vitro* can be drastically altered by a variety of agents with differing physicochemical properties, it is useful to have the additional information provided by an *in vivo* system, as in the present study, as an aid in evaluation of the biologic significance of substances affecting lysosomes. It is possible that the protective effect of cortisone against excess vitamin A, apparently due to increased stability of lysosomes, reflects a fundamental biologic action of this hormone.

Summary

Acute hypervitaminosis A was induced in young rabbits by the oral administration of vitamin A palmitate or vitamin A acid. Gross effects included loss of hair and collapse of the distal third of the rabbits' ears. Histologically, depletion of cartilage matrix was seen, especially of articular and epiphyseal cartilage. These alterations were largely prevented by the simultaneous administration of cortisone. A local protective effect of adrenal corticoids was demonstrated by the intra-articular injection of hydrocortisone in rabbits with hypervitaminosis. The protective effect of the adrenal corticosteroids may be due to an increased stability of lysosomes.

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[*Illustrations follow*]

Legends for Figures

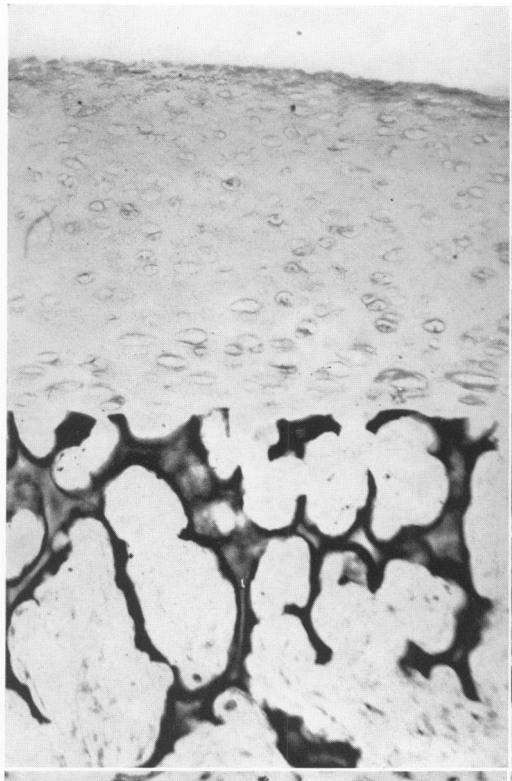
All sections were stained with toluidine blue.

Fig. 1. Rabbit treated with vitamin A, 500,000 units daily for 6 days. The articular cartilage is thin and shows complete loss of metachromatic staining. The bony trabeculae stain normally. × 150.

Fig. 2. Rabbit treated with vitamin A, 500,000 units daily for 6 days, and with cortisone, 12.5 mg. daily. The articular cartilage is of normal thickness and shows normal metachromatic staining. × 150.

Fig. 3. Rabbit shown in Figure 1. The epiphyseal plate is markedly narrowed and devoid of metachromatic staining. × 150.

Fig. 4. Rabbit shown in Figure 2. The epiphyseal plate is slightly thin but shows normal metachromatic staining. × 150.



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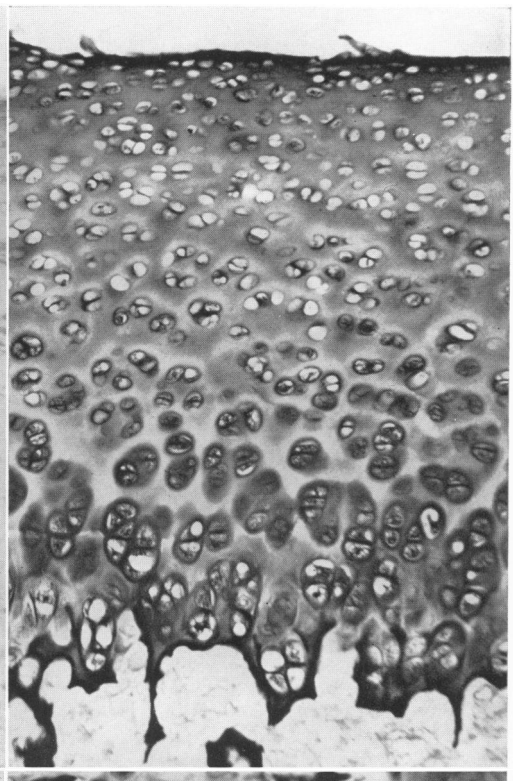

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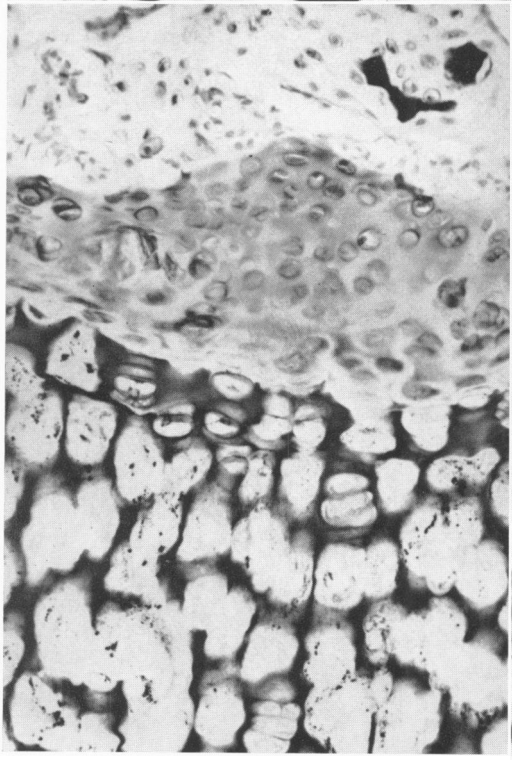

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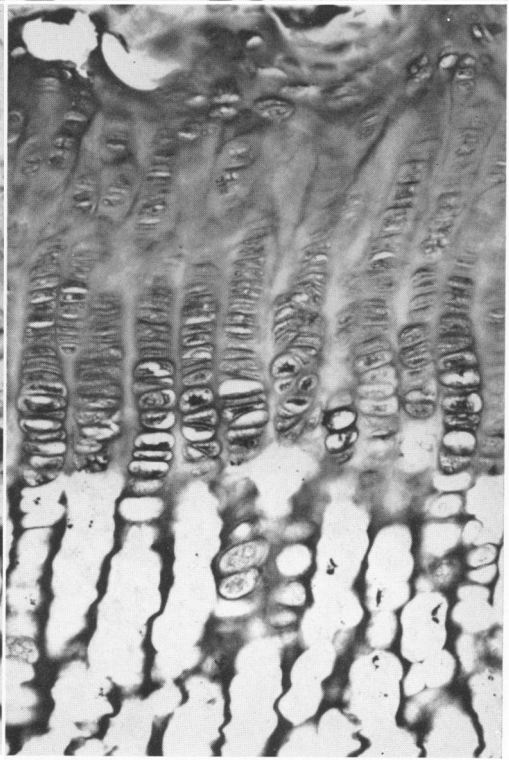

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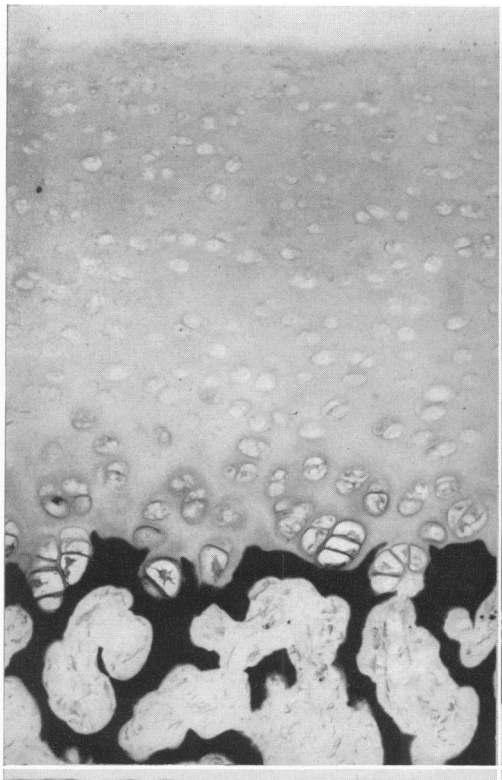
FIG. 5. Rabbit given vitamin A acid, 250 mg. daily for 5 days. The articular cartilage is thin and shows no metachromatic staining. × 150.

FIG. 6. Rabbit given vitamin A acid, 250 mg. daily for 5 days, and concurrently treated with cortisone, 50 mg. daily. The cartilage shows normal staining. × 150.

FIG. 7. The articular cartilage of the left knee (saline-injected joint) from a rabbit given vitamin A palmitate by stomach tube, 750,000 units daily for 3 days. Moderate depletion of cartilage matrix is manifest. × 150.

FIG. 8. The articular cartilage of the right knee (hydrocortisone-injected joint) from the rabbit shown in Figure 7. The articular cartilage exhibits only slight depletion. × 150.



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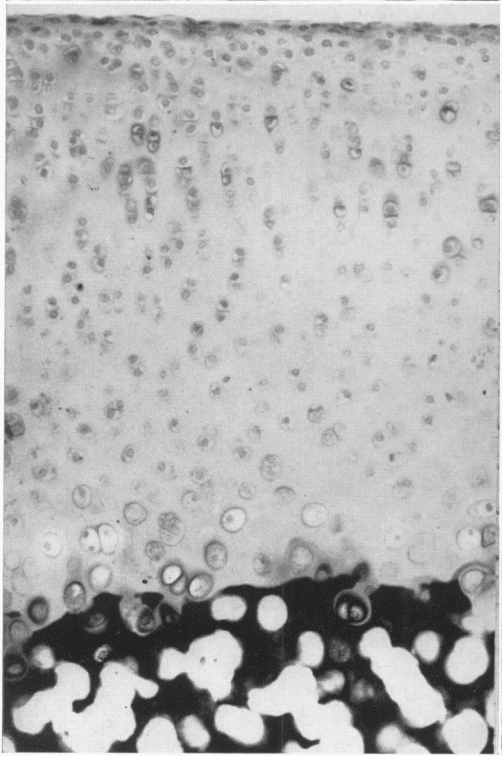

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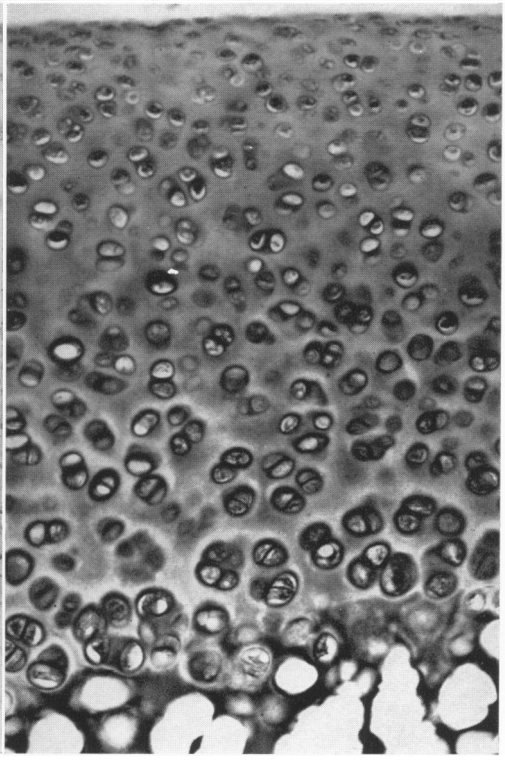

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