VITAMIN A AND ENDOCHONDRAL OSSIFICATION IN THE RAT AS INDICATED BY THE USE OF SULFUR-35 AND PHOSPHORUS-32

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Plates 3 and 4

(Received for publication, March 2, 1954)

Mellanby (1) expressed the opinion that vitamin A influences the activity of osteoblasts and osteoclasts. He focused attention on the deranged calcification in vitamin A deficiency. Wolbach (2), on the other hand, has shown that in vitamin A deficiency the epiphyseal cartilage cells cease to multiply and mature, except cells which have reached the vesicular stage; these latter cells continue to mature and finally disappear, with the result that the epiphyseal cartilage plates are narrower in vitamin A-deficient rats than in normal rats of the same age. The effects of an excessive intake of vitamin A on the skeletons of young rats can be ascribed to the acceleration of normal growth sequences of epiphyseal cartilage cells (2, 3).

The decreased proliferation of epiphyseal cartilage cells in vitamin A-deficient rats suggested that there might be a decreased turnover of the chondroitin sulfate in the cartilage. The results of the experiments described in this paper indicate this to be the case. In addition the experiments indicate that in vitamin A deficiency the mechanisms by which phosphate accumulates in the skeleton are depressed, and that this occurs even sooner than the reduction in uptake of sulfate by epiphyseal cartilage.

Procedure

In the first four sets of experiments, 38 albino rats of the Sherman strain were used. At 21 days of age each of 19 rats was given an intraperitoneal injection of 2 mg. of vitamin A[1] in 0.1 ml. of ethyl laurate, and each of the remaining 19 rats was given a similar injection of ethyl laurate alone. Immediately thereafter, 25 μc. of carrier-free sulfur-35 as sodium sulfate in water[2] was injected intraperitoneally into each rat. 4 to 6 representative animals from each group were sacrificed 7, 24, 48, and 72 hours later. Blood was drawn directly from the heart under deep ether anesthesia and allowed to clot. The serum was separated and stored at 0°C.

[1] This was a commercially available crystalline preparation; the molecular extinction coefficient at 325 to 327 mμ was found to be 46,500, using absolute ethanol as solvent. The value 60,000 at 328 mμ with 95 per cent ethanol as solvent for the vitamin A, has been reported by Holmes, H. N., and Corbet, R. E., *J. Am. Chem. Soc.*, 1937, **59**, 2042.

[2] The sulfur-35 and phosphorus-32 used in this investigation were supplied by the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission. The dosage used was calculated on the basis of the data available from the supplier.

until analyzed, usually the next day. For the collection of the urine, voided during the 24 hours immediately preceding sacrifice, the animals were kept in metabolism cages without food but with water. The urines were stored at 0°C until analyzed.

The sera and urines were analyzed as recently described (4).

The right femur was removed from each rat and stored in a "deep freeze" at about −25°C. until analyzed. For analysis the ends of each femur were separated from the shaft by cutting across the shaft with a pair of scissors, one cut being made just below the lesser trochanter and the other just above the patellar facet. The two ends and the shaft were weighed separately and then placed in porcelain evaporating dishes containing 5 ml. of 0.05 N sodium sulfate. Organic sulfur was oxidized to sulfate by the use of 5 ml. of Denis reagent (5), and then isolated as barium sulfate.

Both tibiae were removed from each rat: the proximal end of one of these was placed in a 3.7 per cent solution (*w/v*) of formaldehyde, and the corresponding end of the other was placed in a 3.7 per cent solution of formaldehyde which had been previously saturated with barium hydroxide. The bones were fixed for 48 hours at 25°C. and then dehydrated by passage through increasing concentrations of ethanol. After passage through xylol they were embedded in paraffin and sectioned at 7 μ. Radioautographs of the sections were prepared as previously described (6). The sections were subsequently stained with 0.1 per cent toluidine blue in 30 per cent ethanol. Additional sections from the tibiae which had been fixed in formaldehyde were also stained with alizarin red as recommended by Dahl (7).

In the second set of experiments, albino rats of the Sherman strain were fed a vitamin A-deficient diet[3] for 42 days, starting with the 28th day of life. On the 70th day of life each of 21 rats (treated) received a single intraperitoneal injection of 2 mg. of vitamin A in 0.1 ml. ethyl laurate, and each of the remaining 20 rats (untreated) received 0.1 ml. of ethyl laurate alone. Immediately thereafter, each of 14 treated and 14 untreated rats was given an intraperitoneal injection of carrier-free sulfur-35 as sodium sulfate in water (0.5 μc. per gm. of body weight). Under deep ether anesthesia, 4 rats from each of the two groups were sacrificed by exsanguination 24 hours later; 5 more from each group were sacrificed at 72 and at 120 hours after injection.

The remaining 6 untreated and 7 treated rats were given an intraperitoneal injection of carrier-free sulfur-35 as sodium sulfate (0.5 μc. per gm. of body weight) 72 hours after administration of ethyl laurate and vitamin A in ethyl laurate, respectively. These animals were sacrificed 24 hours later.

Sera, urines, femurs, and tibiae were taken for analysis as in the first set of experiments.

In the third set of experiments, 62 albino rats of the Sherman strain were maintained on the vitamin A-deficient diet for 42 days, starting with the 28th day of life. At the end of the depletion period, 34 of the rats were given 0.1 ml. of ethyl laurate by intraperitoneal injection, and the remaining 28 rats were given 2 mg. of vitamin A in 0.1 ml. of ethyl laurate. Thereafter, at intervals of time representative animals from each group received labelled sodium sulfate by intraperitoneal injection in a dose of 0.3 μc. of carrier-free sulfur-35 per gm. of body weight. The animals were sacrificed by exsanguination under deep ether anesthesia 12 hours after receiving the isotope. Urine specimens were collected during the 12 hours immediately preceding sacrifice.

Sera, urines, femurs, and tibiae were again analyzed as in the first set of experiments. In addition, after removing most of the musculature, the remainders of the skeletons from the animals in each subgroup were separately pooled. The pelts of the animals were similarly pooled. Separate pools were also formed of all the viscera of the untreated and treated rats.

The tissues in each pool were minced and added to about 10 times their weight of 95 per

[3] The vitamin A-deficient diet was purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

cent ethanol. This ethanol was replaced 24 hours later by an equal volume of fresh 95 per cent ethanol, which after an additional 24 hours was removed by filtration. A large Buchner funnel without filter paper was used. The pools of tissue were separately dried in air for 24 to 48 hours and then for 12 hours at 110°C. Each pool of dried skeletons and dried viscera was then further pulverized by grinding in a mortar with a pestle. Mucopolysaccharide samples were isolated from the tissue samples by an adaptation of the procedure described by Boström (8) for the preparation of chondroitin sulfate from rib cartilage of rats.

For the determination of sulfur-35 in the isolated samples of mucopolysaccharides, weighed portions (about 10 mg.) were each added to 5 ml. of 0.05 N sodium sulfate solution (carrier sulfate). A 10 ml. aliquot of 2.5 N hydrochloric acid and 100 ml. of water were added. The volume was reduced to about 10 ml. by gentle boiling on a hot plate. The water was replaced and boiled away two more times. Finally 200 ml. of water was added and the solution brought to a boil. The sulfate was precipitated by the slow addition of 5 ml. of a 10 per cent barium chloride solution. Gentle boiling was continued until the volume had been reduced to about 100 ml. The precipitate of barium sulfate was collected on a filter paper disk as previously described (9). The radioactivity of each sample was determined with a G-M tube having a mica end-window with a thickness of 1.5 mg./cm.^{2}. The distance from the sample to the end-window was about 2 mm. All values for radioactivity were corrected for decay and self absorption.

The sulfate-sulfur content of the mucopolysaccharide samples was determined after acid hydrolysis of 70 to 100 mg. of sample, the procedure being the same as above except that no carrier sulfate was added. Sulfate was precipitated as barium sulfate and isolated in tared Gooch crucibles. However, in the case of the pelts each pool yielded only 30 to 40 mg. of the mucopolysaccharide preparation; from each pool a 10 mg. portion was added to the same beaker to form a composite sample for sulfate analysis.

The nitrogen content of each mucopolysaccharide sample was determined by micro Kjeldahl analysis (10). The hexuronic acid content was estimated by the procedure described by Dische (11). After each sample had been hydrolyzed as recommended by Einbinder and Schubert (12), the hexosamine contents were determined according to Sørensen (13).

For comparison, 4 rats raised on a diet of Purina dog biscuits (stock diet) to the same age, namely 70 days, were given by intraperitoneal injection 0.3 μc. per gm. of body weight of carrier-free sulfur-35 as sodium sulfate. The animals were sacrificed 12 hours later and their tissues were analyzed as described above.

In the fourth set of experiments, forty albino rats of the Sherman strain were used. They were maintained on the vitamin A-deficient diet from the 28th to the 70th day of life. Each of 20 animals was then given 2 mg. of vitamin A in 0.1 ml. of ethyl laurate by intraperitoneal injection and each of the remaining 20 rats was similarly given ethyl laurate alone. At intervals of time, thereafter, 4 animals from each group received phosphorus-32 as disodium phosphate[3] in water by intraperitoneal injection. The dose was 1 μc. of carrier-free phosphorus-32 per gm. of body weight. 12 hours after administration of the isotope, the animals were sacrificed by exsanguination under deep ether anesthesia. Sera, urines, femurs, and tibiae were taken for analysis.

Proximal ends of the tibiae were placed in a 3.7 per cent solution of formaldehyde which had been previously saturated with magnesium carbonate. The bones were kept in the fixative for 48 hours at 25°C. and then were dehydrated in increasing concentrations of ethanol, starting with a 30 per cent concentration. The ethanol solutions were saturated with magnesium carbonate before use. After passage through two changes of xylol, the bones were imbedded in paraffin and sectioned at 7 μ. Radioautographs of the sections were prepared as previously described (6). The sections were subsequently stained with alizarin red as recommended by Dahl (7).

Each of 4 normal rats, 70 days old, previously maintained on a stock diet of Purina dog biscuits, was given 1 μc. of phosphorus-32, as disodium phosphate, per gm. of body weight by intraperitoneal injection, 12 hours before sacrifice. Sera, urines, femurs, and tibiae of these animals were analyzed as were those from the vitamin A-deficient rats.

For the determination of phosphorus-31 and phosphorus-32 contents, each femur was divided into ends and shafts as described above. The combined ends were weighed separately from the shaft. The samples were heated in 50 ml. pyrex beakers at 500°C. in a muffle furnace for 6 hours. To the cooled beaker, 2 ml. of 8 N nitric acid was added and evaporated with heat. The beaker was reheated at 400°C. for 15 minutes. After cooling, 1 ml. of concentrated hydrochloric acid was added and evaporated. The residue was dissolved in 10 ml. of 3 N hydrochloric acid and transferred to a 25 ml. volumetric flask. After diluting to volume with water, an aliquot, 2 ml. in the case of the femoral ends and 5 ml. in the case of the femoral shafts, was further diluted to 25 ml. with water. The phosphorus-32 contents of the solutions so prepared were determined with a dipping G-M tube and a scaler. All values were corrected for decay. The concentrations of total phosphorus in the solutions were determined by an adaptation of the method of Fiske and Subbarow (14). A procedure similar to the above has been used by McAuliffe (15) for the determination of phosphorus-32 in plant materials.

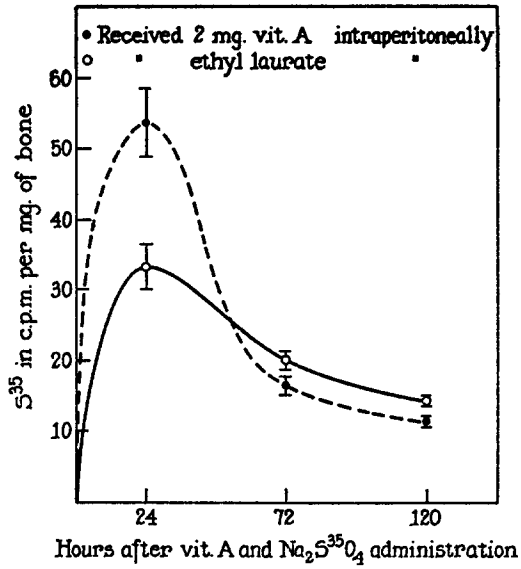
One ml. of each of the sera was added to 9 ml. of a 5 per cent solution of trichloracetic acid in a centrifuge tube. After mixing and centrifuging, a 1 ml. aliquot of the supernate was transferred to a calibrated digestion tube and digested as recommended by Fiske and Subbarow (14). The phosphorus-32 and total phosphorus concentrations were determined as described above.

The inorganic phosphorus concentration in each urine specimen was determined by an adaptation of the method of Fiske and Subbarow (14). The phosphorus-32 contents were assayed with the dipping G-M tube and scaler after suitable dilution of the urines with 0.1 N hydrochloric acid. Corrections for decay of radioactivity were made.

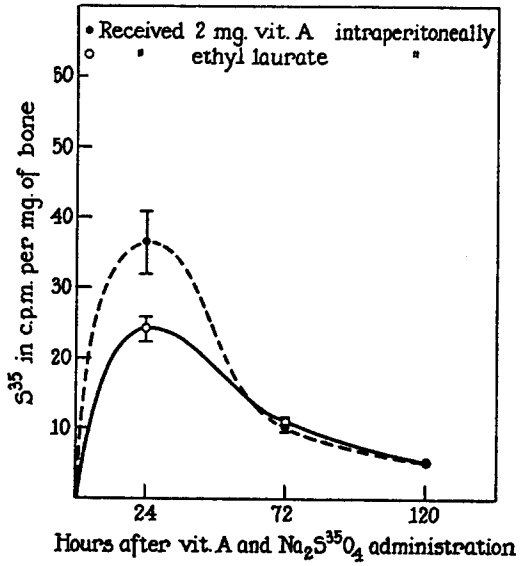
RESULTS

The Lack of Effect of Vitamin A When Given to Normal Weanling Rats on an Adequate Diet.—The 21-day-old stock rats, each of which received 2 mg. of vitamin A in ethyl laurate, apparently disposed of the concurrently administered sulfate as did their litter mates to whom only ethyl laurate was given. The sulfur-35 and inorganic sulfate levels in the sera, and the fractions of the dose of sulfur-35 which were excreted in the urines were similar. Vitamin A had no effect on the concentrations of sulfur-35 in the femurs, nor on the autographs produced by sections of tibiae. On microscopic examination of the tibial sections no effect of vitamin A on the epiphyseal cartilage was noted.

The Immediate Effects of Administering Vitamin A to Deficient Rats.— The sulfur-35 levels in the sera and the fractions of the dose of the isotope excreted in the urines in the second set of experiments were also similar whether the deficient animals were or were not given vitamin A. Between the 24th and 120th hour after administration of the vitamin, however, the concentration of inorganic sulfate-sulfur in the sera decreased from a value of about 2.5 mg. per cent to a value of about 1.8 mg. per cent. The value for the concentration of inorganic sulfate-sulfur in the sera of normal stock rats of the same age was found to be 2.0 ± 0.1 mg. per cent.



TEXT-FIG. 1. Effect of vitamin A on the disposition of sulfur-35 in the ends of femurs in vitamin A-deficient rats. Each rat received 0.5 μc. of carrier-free sulfur-35 as sodium sulfate per gm. of body weight at the same time that either ethyl laurate or 2 mg. of vitamin A in ethyl laurate were administered intraperitoneally. The standard deviation of each average value is indicated by a vertical bar.



TEXT-FIG. 2. Effect of vitamin A on the disposition of sulfur-35 in the shafts of femurs in vitamin A-deficient rats. The legend under Text-fig. 1 also applies here.

The concentration of sulfur-35 in the femurs of vitamin A-treated rats was higher 24 hours after administration and subsequently decreased more rapidly than in the femurs of the untreated rats, Text-figs. 1 and 2.

In Table I are given the averaged concentrations of sulfur-35 found in the femoral ends and shafts of rats that received either vitamin A or only ethyl laurate 72 hours before administration of sulfur-35. It can be seen that in the 24 hours following injection of the isotope more of it was concentrated in the femurs of the rats pretreated with vitamin A than in the femurs of their untreated litter mates.

Radioautographs produced by sections of tibiae fixed in a solution of formaldehyde are shown as Figs. 1 through 8. It can be seen that the epiphyseal cartilage of the tibiae removed from rats 24 hours after the concurrent ad-

TABLE I

Effect of Vitamin A on the Concentration of Sulfur-35, Administered as Sodium Sulfate, in Femurs of Vitamin A-deficient Rats

Tissue	A-deficient rats	A-deficient rats given vitamin A
	c.p.m./*mg. of bone*	c.p.m./*mg. of bone*
Femoral ends	36.9 ± 1.2	54.9 ± 2.4
Femoral shaft	29.3 ± 3.0	36.2 ± 2.4

After the rats had been maintained on a vitamin A-deficient diet for 42 days, some of the rats were given 2 mg. each of vitamin A in ethyl laurate and others were given ethyl laurate alone by intraperitoneal injection. 72 hours later each rat was given an intraperitoneal injection of carrier-free sulfur-35 as sodium sulfate in a dose of 0.5 μc. per gm. of body weight. The animals were sacrificed 24 hours later. The values in the table are averages and standard deviations for groups of 6 untreated and 7 treated rats.

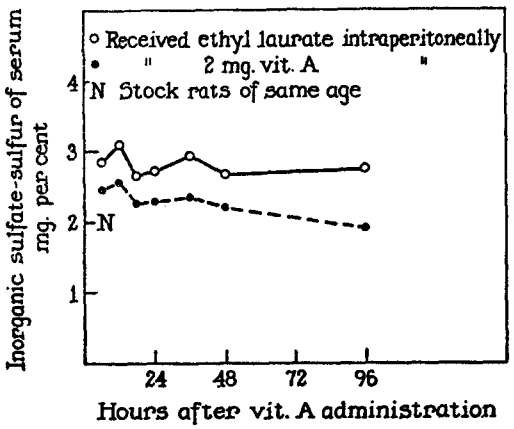
ministration of vitamin A and sulfur-35, Fig. 1, elicited a stronger reaction on the photographic film than did the epiphyseal cartilage of the tibiae from the untreated rats, Fig. 5. The converse was observed in the case of the tibiae removed 72 and 120 hours after injection, compare Fig. 2 with 6 and Fig. 3 with 7, respectively. Similar results were obtained in radioautographs of the diaphyses.

It can also be seen that, in agreement with the data of Table I, the radioautographic image produced by sections of tibiae from rats given vitamin A 96 hours and sulfur-35 24 hours before sacrifice is darker, Fig. 4, than that produced by sections of tibiae from the untreated animals, Fig. 8. In addition, the epiphyseal plate of the tibiae was broader in those animals which had been given vitamin A.

Radioautographs produced by sections of tibiae fixed in a solution of formaldehyde saturated with barium hydroxide are shown as Figs. 9 through 16. In animals given vitamin A and labelled sulfate progressively more sulfur-35 was deposited at the epidiaphyseal junction than in the same region of the tibiae

from the untreated vitamin A-deficient rats, Figs. 9, 10, and 11 compared with Figs. 13, 14, and 15, respectively. The effect of vitamin A on the concentration of sulfur-35 in the tibiae is again indicated by a comparison of Fig. 12 with Fig. 16.

The Delayed Effects of a Single Dose of Vitamin A Given to Deficient Rats as Reflected in the Uptake of Sulfur-35.—Neither the level of total sulfur-35 in the sera nor the fraction of the dose of sulfur-35 excreted in the urine by the vitamin A-treated rats was strikingly different from the corresponding values for untreated rats. In contrast, the concentration of inorganic sulfate-sulfur in the serum was found to have decreased from an average of 2.5 mg. per cent to 1.9



TEXT-FIG. 3. Effect of a single dose of vitamin A on the concentration of inorganic sulfate-sulfur in the sera of vitamin A-deficient rats. Each value is the average for a group of 4 or 5 rats. The abscissa indicates the time after vitamin A administration at which sulfur-35 was administered to the rats. For the purpose of obtaining data recorded in other figures the sera were collected 12 hours after injection of the isotope.

mg. per cent between the 18th and 108th hour after vitamin A administration, Text-fig. 3.

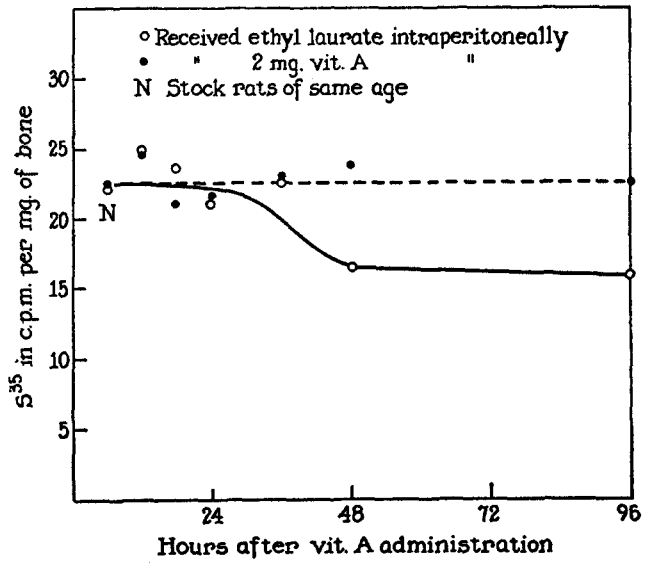
The concentration of sulfur-35 in the femoral ends but not in the femoral shafts was found to be affected by the prior administration of vitamin A, Text-figs. 4 and 5.

Radioautographs produced by sections of tibiae fixed in a solution of formaldehyde are shown as Figs. 17 through 24. It can be seen that sections of tibiae from the untreated animals produced radioautographs that were similar to those produced by sections of tibiae from vitamin A-treated animals if the animals were examined at the end of the 42 day depletion period, or shortly thereafter, compare Figs. 21 and 22 with Figs. 17 through 20. The magnitude of the radioautographic reaction was similar to that produced by sections of tibiae from normal stock rats of the same age. Continued maintenance of the untreated rats

on the deficient diet was reflected in the production by the epiphyseal cartilage plate of a radiographic image of diminished density and width, Figs. 23 and 24.

On examination of the radioautographs produced by the sections of tibiae fixed in a solution of formaldehyde saturated with barium hydroxide no difference was made out between those produced by the tibial sections from the vitamin A-treated or untreated rats.

The above data and those presented graphically in Text-fig. 6 show that the metabolism of chondroitin sulfate in the skeleton is under the influence of

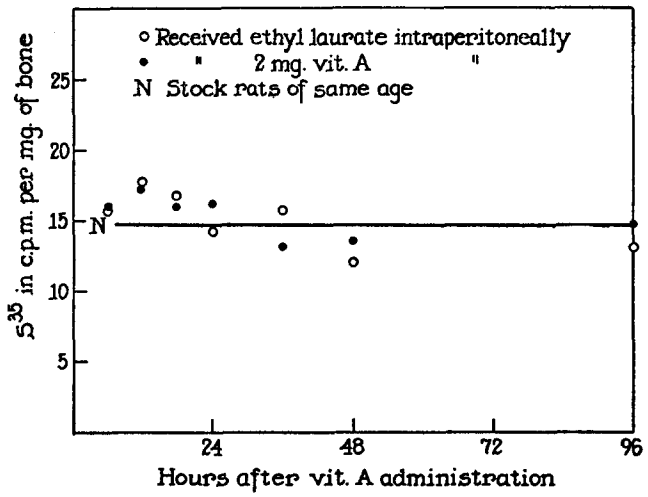


TEXT-FIG. 4. Effect of vitamin A on the concentration of sulfur-35 in the ends of femurs from vitamin A-deficient rats. Each rat received 0.3 μc. of carrier-free sulfur-35, as sodium sulfate, per gm. of body weight at intervals of time after the administration of vitamin A, (or of ethyl laurate only) as indicated on the abscissa. The animals were sacrificed 12 hours later. The values plotted are the averages obtained from 4 or 5 rats.

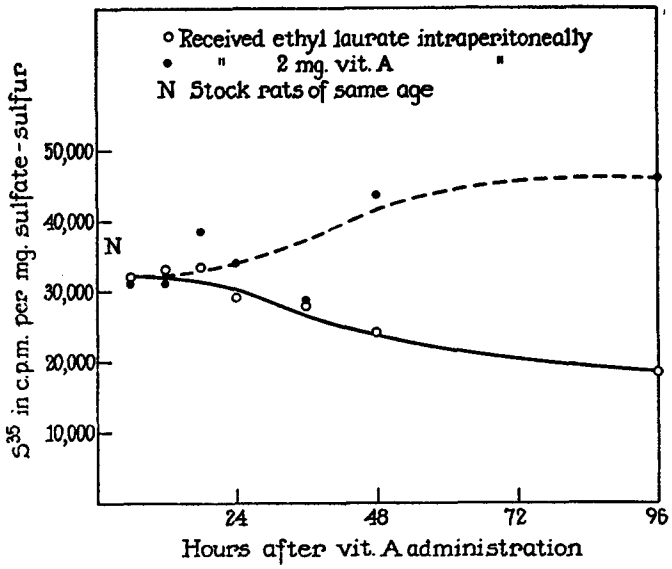
vitamin A, for the specific activity of the sulfur-35 in the chondroitin sulfate samples isolated from the skeletons is affected by the administration of this vitamin.

Vitamin A also exerts an influence on the metabolism of the sulfomucopolysaccharides of the rat skin as shown in Text-fig. 7, and it probably has an influence on the mucoitin sulfuric acid of the gastrointestinal tract since the respective specific activities of the samples isolated from the viscera of the untreated deficient rats, vitamin A-treated rats, and normal rats were found to be 85,300, 94,100, and 111,600-values which are significantly different.

The Delayed Effects of a Single Dose of Vitamin A Given to Deficient Rats as Shown in the Uptake of Phosphorus-32.—The concentrations of trichloracetic acid–soluble phosphorus and phosphorus-32 in the sera, and the values for the

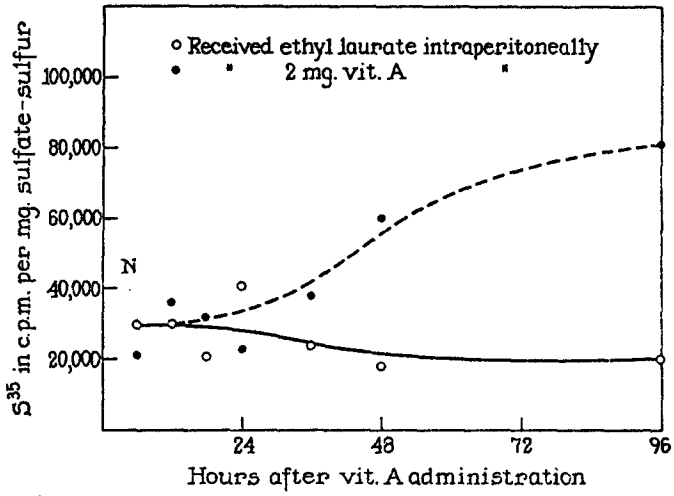


TEXT-FIG. 5. Effect of vitamin A on the concentration of sulfur-35 in the shafts of femurs from vitamin A-deficient rats. The legend under Text-fig. 4 also applies here.



TEXT-FIG. 6. Concentration of sulfur-35 in chondroitin sulfate samples isolated from the skeletons of vitamin A-deficient rats. Each rat received by intraperitoneal injection 0.3 μc. of carrier-free sulfur-35, as sodium sulfate, per gm. of body weight at intervals of time after the administration of vitamin A (or of ethyl laurate only), as indicated on the abscissa. The animals were sacrificed 12 hours later. The isolated chondroitin sulfate samples resembled each other closely in composition. Their composition did, however, deviate from that usually ascribed to chondroitin sulfate. On analysis about 3 per cent of sulfate-sulfur, about 5 per cent of nitrogen, about 26 per cent of hexuronic acid, and about 30 per cent of hexosamine were found in these samples.

specific activity of the inorganic phosphorus fractions in the urines, were essentially the same in the deficient rats as in those which had been given vitamin A. The specific activity of the phosphorus-32 in the femoral ends and shafts of the rats given vitamin A 48 hours or more before administration of the labelled phosphate was found to be higher than that in the femurs of the untreated rats, Text-fig. 8, and approached the values found in the same regions of the femurs from normal rats of the same age on a stock diet, namely 10,000 and 4,200 C.P.M. per mg. of phosphorus in femoral ends and shafts, respectively. Not only



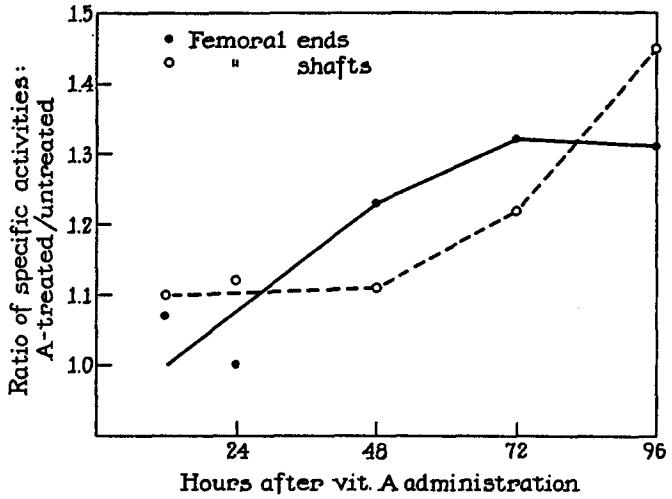
TEXT-FIG. 7. Concentration of sulfur-35 in samples of mucopolysaccharides isolated from the skins of vitamin A-deficient rats. Each rat received by intraperitoneal injection 0.3 μc. of carrier-free sulfur-35, as sodium sulfate, per gm. of body weight. The isotope was injected at intervals of time after the administration of vitamin A (or of ethyl laurate only). The time at which the isotope was administered is given on the abscissa. The animals were sacrificed 12 hours later. On analysis, the isolated mucopolysaccharide samples were shown to contain about 2 per cent of sulfate-sulfur, about 9 per cent of nitrogen, about 15 per cent of hexuronic acid, and about 18 per cent of hexosamine.

was more phosphorus-32 taken up by the femurs, the concentration of total phosphorus also rose: The concentrations of phosphorus in the femurs of the rats given vitamin A solution were found to be intermediate (4.50 and 6.75) between those of the vitamin A-deficient (3.70 and 6.10) and normal rats (5.42 and 8.02). The values are given as per cent of phosphorus in the ends and shafts, respectively, and in the case of the vitamin A-treated rats are the values found 108 hours after vitamin administration.

The radioautographic evidence for the effect of vitamin A on the deposition of phosphorus in the tibiae of vitamin A-deficient rats is reproduced in part in Figs. 25–32. It can be seen that sections of the tibiae removed from rats that had received the vitamin at least 48 hours before the labelled phosphate gave

more pronounced radioautographic reactions, Figs. 26 to 28, than did the sections of the tibiae either from animals given the phosphorus-32 sooner, Fig. 25, and from untreated vitamin A-deficient rats, Figs. 29 through 32.

The extent to which the sections stained with alizarin red was correlated with the density of the autographs they produced: an intense radioautographic reaction invariably accompanied a deep stain, particularly at the epidiaphyseal junction. It is of interest that in the third set of experiments an intensification of staining with alizarin red occurred also in the sections removed from the rats 36 to 48 hours after vitamin A administration.



TEXT-FIG. 8. Effect of vitamin A on the concentration of phosphorus-32 in the femurs of vitamin A-deficient rats. Each rat received 1 μc. of carrier-free phosphorus-32, as disodium phosphate in water, per gm. of body weight. The isotope was injected intraperitoneally at intervals of time after the administration to each rat of 2 mg. of vitamin A in ethyl laurate (or of ethyl laurate only). The time at which the phosphorus-32 was administered is given on the abscissa. The animals were sacrificed 12 hours later. The concentrations of the phosphorus-32 in the femurs were calculated as specific activities (counts per minute per milligram of total phosphorus).

DISCUSSION

In regions where endochondral ossification occurs there is an arrangement of cartilage cells in rows parallel to the long axis of the bone. Prior to calcification the cartilage cells enlarge and then degenerate. The multiplication, maturation, and degeneration of the cells in the cartilage plate at epidiaphyseal junctions continues until growth in length of the bone is completed. Supposedly, a function of the cartilage cells is the elaboration of the matrix in which they are imbedded. Chondroitin sulfate is part of this matrix. Therefore, since there is in vitamin A deficiency, a characteristic arrest of this sequence of normal cellular maturation, one might find a decreased synthesis of chondroitin sulfate

in vitamin A-deficient animals as compared with that in normal or in deficient animals given vitamin A. The experiments detailed in this paper indicate that such a decreased synthesis of chondroitin sulfate actually occurs in vitamin A-deficient rats. Further, as a result of the administration of vitamin A the rate at which chondroitin sulfate is synthesized can be restored to, maintained at, or even increased above the rate of synthesis in normal rats. A depression in the synthetic mechanism was demonstrated only after the rats were unmistakably deficient, Text-figs. 4 and 6; at this stage the administration of vitamin A was promptly reflected in increased rate of uptake of S^{35}, (Text-fig. 2 and Figs. 1 and 5) presumably due to accelerated synthesis of chondroitin sulfate.

Vitamin A also influences the metabolism of phosphorus in the rat skeleton, Text-fig. 8 and Figs. 17 to 32. The uptake of phosphorus by femurs and tibiae of vitamin A-deficient rats was observed to be less than the uptake by these bones in normal rats. The administration of vitamin A to the animals was reflected in an unmistakably increased uptake of phosphorus in the skeleton, particularly in regions of most active calcification. This effect, however, was not apparent until about 48 hours after administration of the vitamin A.

One would like to compare the two sets of experiments in which sulfur-35 and phosphorus-32 were used under similar conditions. It would be of interest to know whether in the skeletons of animals deprived of vitamin A phosphorus metabolism is depressed before a decreased metabolism of sulfate occurs. The radioautographs reproduced in Figs. 17 to 32 and the radiochemical data, Text.-figs. 4 and 8, suggest that this is probably the case. The fact that the alizarin stained tibial sections from the third set of experiments resembled those in the fourth set gives further weight to this suggestion.

After the administration of vitamin A to deficient rats not only rate of synthesis but also the rate of degradation of chondroitin sulfate is accelerated, Text-figs. 1 and 2 and Figs. 1 to 16. In the photographs of Figs. 1 to 16 there is also a suggestion that the sulfur of chondroitin sulfate is deposited in the newly formed spongy bone as the latter replaces cartilage. The sulfur-35–containing material deposited in these recently calcified regions has not been identified; it is more soluble than chondroitin sulfate in a 3.7 per cent solution of formaldehyde but less soluble than the chondroitin sulfate in a 3.7 per cent solution of formaldehyde previously saturated with barium hydroxide.

Skin lesions have been reported as an early manifestation of vitamin A deficiency in experimental animals and in man (16–18). The sebaceous and sweat glands, the hair, and the nails are affected; the skin appears dry, scaly, and shrivelled. In this connection it is of interest that the rate at which sulfate-sulfur is incorporated into the sulfomucopolysaccharides of the skin of vitamin A-deficient rats is slightly lower than the rate of incorporation observed in normal rats, Text-fig. 7. Administration of vitamin A is followed by increase of the rate to that in normal rats or even to a rate above normal.

SUMMARY

The administration of vitamin A to vitamin A-deficient rats resulted in a decreased concentration of inorganic sulfate-sulfur in the serum from a value of 2.5 mg. per cent to 1.8 mg. per cent, the latter being close to the value of 2.0 mg. per cent found in normal rats of the same age.

The uptake of sulfate and phosphate by femurs and tibiae of vitamin A-deficient rats was less than that in normal rats of the same age. An increased uptake followed the administration of vitamin A: radioautography indicated that in the case of sulfate, its uptake was particularly increased in the epiphyseal cartilage; an increased uptake of phosphate was particularly evident in the diaphysis immediately adjacent to the epiphyseal cartilage plate.

The specific activity of the sulfate-sulfur in the chondroitin sulfate samples isolated from the skeletons of vitamin A-deficient rats fell progressively as the deficiency continued. Following administration of vitamin A, the specific activity approached and exceeded the value given by the sample from the skeletons of normal rats of the same age.

A substantial increase was found in the value of the specific activity of the sulfate-sulfur of sulfomucopolysaccharides isolated from skins of vitamin A-deficient rats that had been given vitamin A.

Following administration of vitamin A to rats deficient in this vitamin, an increased accumulation of some sulfur-containing material was found in regions of active calcification.

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EXPLANATION OF PLATES

PLATE 3

Radioautographs produced by sections of the proximal ends of tibiae fixed for 48 hours at 25°C. in a 3.7 per cent solution of formaldehyde (Figs. 1 through 8) or in a 3.7 per cent solution of formaldehyde previously saturated with barium hydroxide (Figs. 9 through 16). Kodak contrast process film was exposed to the sections for 2 and 4 weeks, respectively. Each of the rats received 0.5 μc. of sulfur-35 as sodium sulfate per gm. of body weight.

FIGS. 1, 2, and 3. Photographs of radioautographs produced by sections of tibiae removed from vitamin A-deficient rats 24, 72, and 120 hours after the concurrent intraperitoneal injection of 2 mg. of crystalline vitamin A in ethyl laurate and sulfur-35. × 3.7.

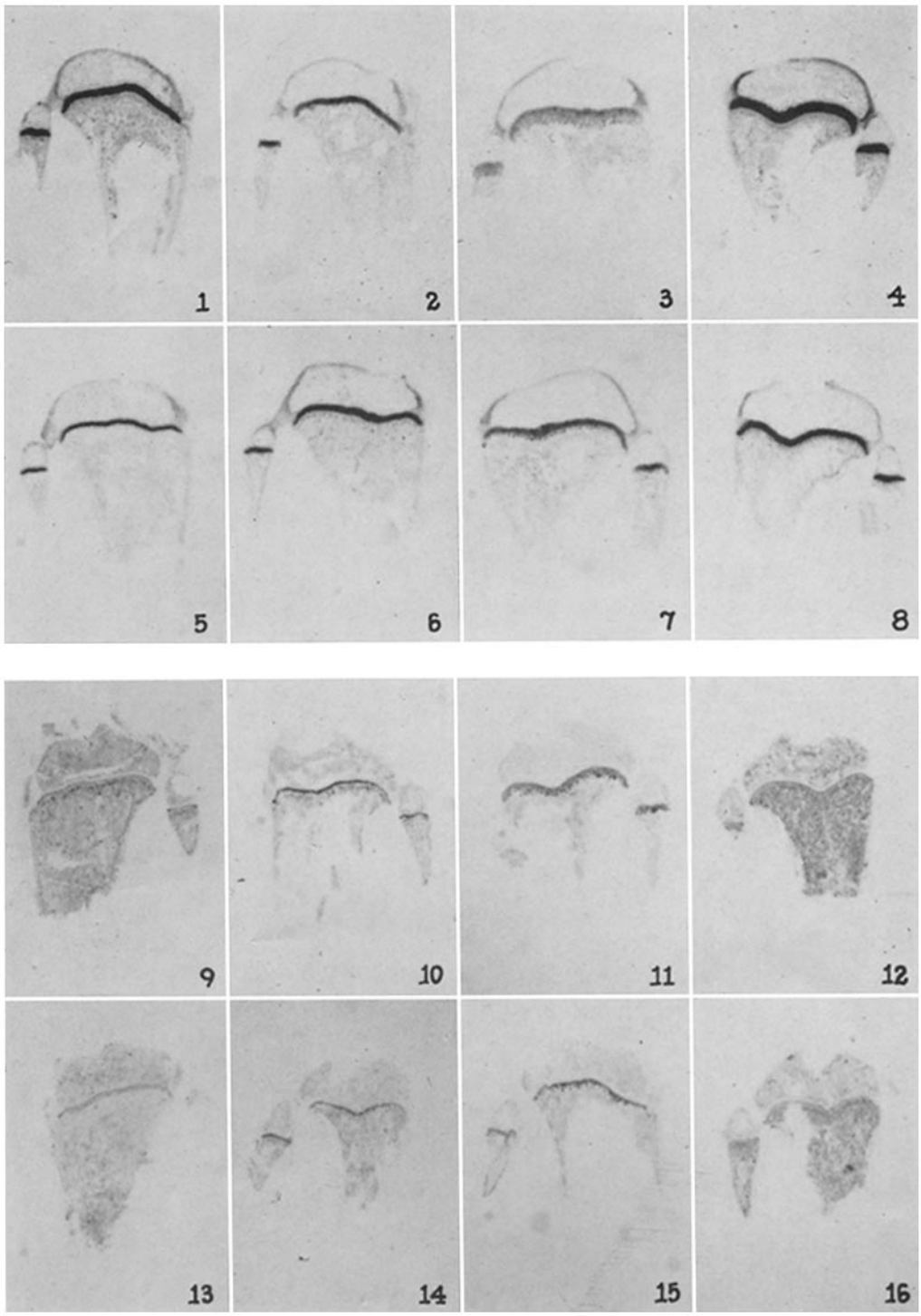
FIGS. 5, 6, and 7. To be compared with Figs. 1, 2, and 3, respectively. They are from vitamin A-deficient rats that received concurrently injections of ethyl laurate and sulfur-35. × 3.7.

FIGS. 4 and 8. Photographs of radioautographs produced by sections of tibiae from vitamin A-deficient rats 24 hours after the intraperitoneal injection of sulfur-35. In one case (Fig. 4) 2 mg. of vitamin A in ethyl laurate and in the other (Fig. 8) only ethyl laurate was injected 72 hours before administration of the isotope. × 3.7.

FIGS. 9, 10, and 11. Photographs of radioautographs produced by sections of tibiae removed from vitamin A-deficient rats 24, 72, and 120 hours after the concurrent intraperitoneal injection of 2 mg. of crystalline vitamin A in ethyl laurate and sulfur-35. × 3.7.

FIGS. 13, 14, and 15. To be compared with Figs. 9, 10, and 11, respectively. The former are from the vitamin A-deficient rats that were given ethyl laurate only and sulfur-35. × 3.7.

FIGS. 12 and 16. Photographs of radioautographs produced by sections of tibiae from vitamin A-deficient rats 24 hours after the intraperitoneal injection of sulfur-35. 2 mg. of vitamin A in ethyl laurate were given in one case (Fig. 12) and only the ethyl laurate in the other, 72 hours before administration of the isotope. × 3.7.



(Dziewiatkowski: Vitamin A and endochondral ossification)

PLATE 4

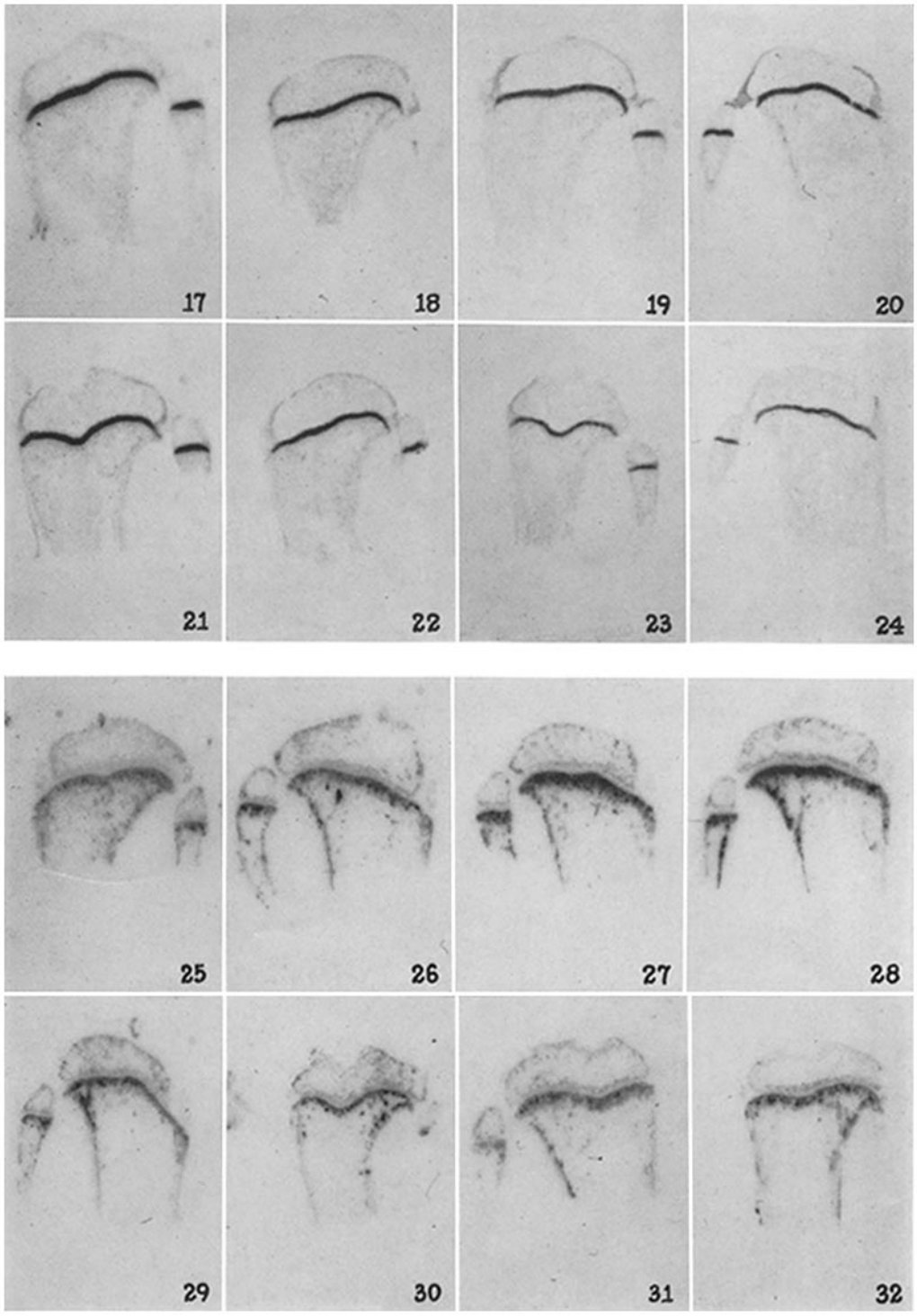
Radioautographs produced by sections of the proximal ends of tibiae fixed for 48 hours at 25°C. in a 3.7 per cent solution of formaldehyde (Figs. 17 through 24) or in a 3.7 per cent solution of formaldehyde previously saturated with magnesium carbonate. (Figs. 25 through 32). Kodak contrast process ortho film was exposed to the sections for 2 weeks and 1 week, respectively. The animals received each per gm. of body weight 0.3 μc. of sulfur-35 as sodium sulfate (Figs. 17 through 24) or 1 μc. of phosphorus-32 as disodium phosphate (Figs. 25 through 32).

FIGS. 17, 18, 19, and 20. Produced by sections of tibiae removed from vitamin A-deficient rats that had received each 2 mg. of vitamin A. Sulfur-35 was injected 12, 24, 48, and 96 hours after vitamin administration, respectively. The tibiae were removed 12 hours after the administration of the sulfur-35. $\times$ 3.7.

FIGS. 21, 22, 23, and 24. To be compared with Figs. 17, 18, 19, and 20, respectively. They are from vitamin A-deficient rats that received ethyl laurate only and sulfur-35.

FIGS. 25, 26, 27, and 28. Produced by sections of tibiae removed from vitamin A deficient rats that had received each 2 mg. of vitamin A. Phosphorus-32 was injected 12, 24, 48, and 96 hours after administration of the vitamin, respectively. The tibiae were removed 12 hours after injection of the phosphorus-32. $\times$ 3.7.

FIGS. 29, 30, 31, and 32. To be compared with Figs. 25, 26, 27, and 28 respectively. They are from vitamin A-deficient rats that received ethyl laurate only and phosphorus-32. $\times$ 3.7.



(Dziewiatkowski: Vitamin A and endochondral ossification)