EFFECT OF AGE ON SOME ASPECTS OF SULFATE METABOLISM IN THE RAT

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Plates 6 and 7

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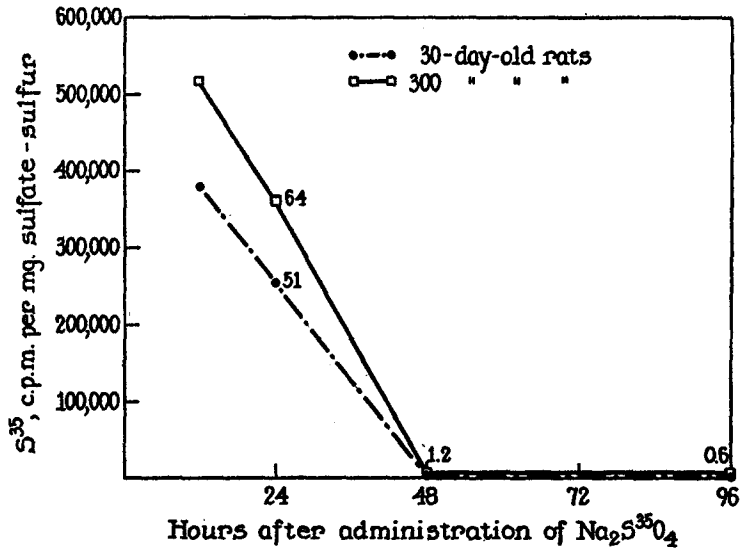
Sulfur which is ingested as sulfate or oxidized to this form by animals is excreted rapidly and to a large extent as sulfate (1–5). About 70 to 80 per cent of an administered dose of (sodium) sulfate is excreted in the urine and feces within 24 hours by adult rats. By the end of 120 hours about 95 per cent of the dose is thus eliminated. Although sulfate-sulfur can be used in the synthesis of cystine in the rat, probably by the bacterial flora of the intestinal tract, the amount of sulfate-sulfur which is converted to cystine-sulfur is negligibly small (6, 7). Most of the sulfate which is retained in the tissues is held there in esterified form (8, 9). Samples of chondroitin sulfate containing sulfur-35 have been isolated from the cartilage of knee joints from young rats (10) and from the rib cartilage of adult rats (11) following administration of S^{35}-labelled sodium sulfate. The incorporation of S^{35}-labelled sulfate into the mucopolysaccharides of rat skin has also been demonstrated (12). In fact, in using radioautography it has been found that tissues in which sulfomucopolysaccharides are present take up more S^{35}-labelled sulfate than tissues from which such polysaccharides are absent (13).

Studies on the uptake *in vitro* of sulfate-sulfur by embryonic chick tissues (14) and by mouse tissues (15), as well as a study *in vivo* of sulfate-sulfur metabolism in the rat fetus (16) indicate that age has an effect on the over-all metabolism of sulfate-sulfur in animals. To better define this effect of age it seemed desirable to investigate the metabolism of sulfate-sulfur in the mucopolysaccharides of the skeletons, pelts, and viscera of rats of different ages. This paper is a report of such a study, in which the sulfur-35 concentration was determined in mucopolysaccharides isolated from various tissues at intervals of time following administration of carrier-free sulfur-35 as sodium sulfate. By use of radioautography not only was one able to confirm the results of the radiochemical assay on the samples isolated from the skeletons but also detailed information was obtained regarding localization of sulfur-35 in humeri. Observations on the association of sulfate-sulfur with serum proteins are also presented.

EXPERIMENTAL

Eighty-eight rats 10 days old, 43 rats 30 days old, and 24 approximately 300 days of age received each an intraperitoneal injection of carrier-free sulfur-35 as sodium sulfate in water.[1] The dose was 0.3 μc.[2] per gm. of body weight. A representative number of animals of each age group were sacrificed by exsanguination under deep ether anesthesia 12, 24, 48, and 96 hours

TEXT-FIG. 1. Sketch of a rat femur. The broken lines indicate where the bone was divided into shaft and ends prior to analysis for sulfur-35 concentration.



TEXT-FIG. 2. Excretion of sulfur-35 as total sulfate-sulfur in rat urine following the intraperitoneal administration of labelled sodium sulfate. Each of the rats used was given 0.3 μc. of carrier-free sulfur-35 per gm. body weight and the urine was collected during the 24 hours preceding sacrifice of the animals. The numbers next to the points give the percentage of the total dose which was excreted during the 24 hour collection period.

after administration of the isotope. The blood was drawn directly from the heart and allowed to clot; the serum was separated, pooled with like samples, and set aside at 0°C. until analyzed. Urines for the 24 hour period just prior to sacrifice were also collected, pooled, and stored at 0°C. until analyzed.

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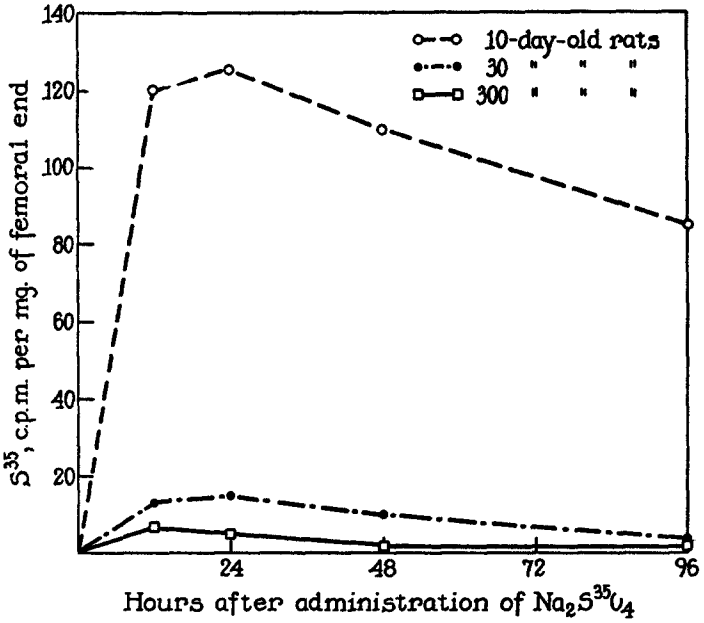
[1] The sulfur-35 used in this investigation was supplied by the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission.

[2] The dose was calculated to the date of shipment on the basis of the assay of radioactivity by the supplier.

The humeri and femurs were removed from 4 animals in each of the 12 groups. The femurs were stored in a "deep freeze" at about −25°C. until analyzed. One humerus from each of the rats was placed in a 3.7 per cent solution of formaldehyde (*w/v*) and the other humerus was placed in a 3.7 per cent solution of formaldehyde previously saturated with barium hydroxide. The bones were kept in the fixative for 48 hours at 25°C. and then dehydrated by passage through increasing concentrations of ethanol and finally xylol. They were imbedded in paraffin and sectioned at 7 μ. Radioautographs of the sections were prepared on Kodak contrast process ortho film as previously described (17).



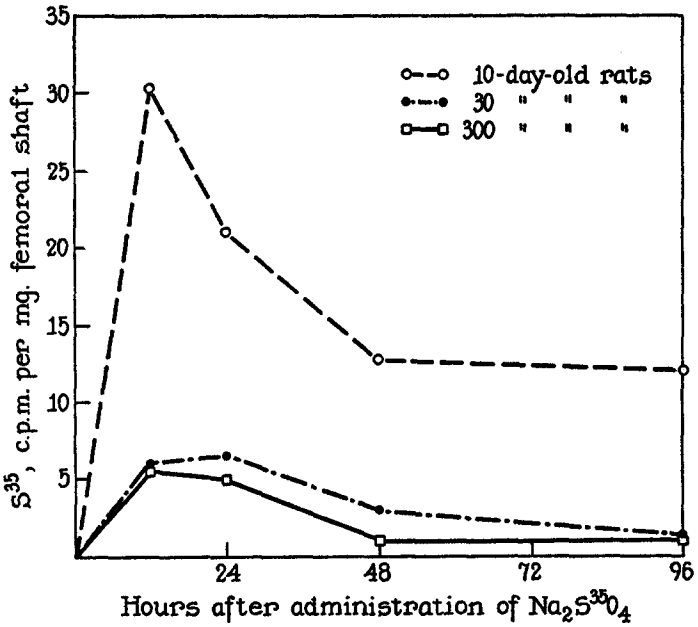
TEXT-FIG. 3. Concentration of sulfur-35 in the ends of femurs removed from rats at intervals of time following the intraperitoneal administration of labelled sodium sulfate. Each of the rats used was given 0.3 μc. of carrier-free sulfur-35 per gm. body weight. The values plotted are the mean values of four separate determinations.

The femurs were divided into ends and shaft by cutting with a pair of scissors as shown by the dashed lines in Text-fig. 1. The shaft and combined ends of each femur were weighed separately and then placed in porcelain evaporating dishes containing 5 ml. of 0.05 N sodium sulfate. The samples were oxidized by the use of the reagent of Denis (18). The sulfate was isolated as barium sulfate.

The pelts of the animals in each group were pooled. Similar pools were made of the viscera and the remainders of the skeletons which were freed of most of their musculature before use. In each of the pools the tissues were minced and added to about 10 times their weight of 95 per cent ethanol. The mixtures were shaken 10 to 12 times during the following 24 hours while being kept at room temperature. The ethanolic extracts were removed by decantation and discarded. Extraction for 24 hours was repeated with fresh portions of ethanol. Most of the alcohol was removed from the tissues by filtration, using a large Buchner funnel. The alcoholic extracts were again discarded. The tissues were dried by exposure to air at room

temperature for 24 hours followed by heating to 110°C. for 12 hours. Each pool of dried tissue, except that of pelts, was passed through a meat grinder and the resultant coarse powders were weighed. Mucopolysaccharides containing sulfur-35 were isolated from the dried, ground tissue samples by an adaptation of the procedure used by Boström (11) for the isolation of chondroitin sulfate from rat rib cartilage.

For the determination of sulfur-35 weighed portions of the mucopolysaccharide samples were each added to 5 ml. of a 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



TEXT-FIG. 4. Concentration of sulfur-35 in the shafts of femurs removed from rats at intervals of time following the intraperitoneal injection of labelled sodium sulfate. Each of the rats used was given 0.3 μc. of sulfur-35 per gm. body weight. The values plotted are the mean values of four separate determinations.

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 (16). The radioactivity of each sample was determined with an end-window G-M tube and a Tracerlab "1000" scaler. The mica end-window had 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 about 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.

The nitrogen content of the mucopolysaccharide samples was determined by micro Kjeldahl analysis (19); the hexuronic acid content was estimated according to the procedure proposed by Dische (20); the hexosamine content was determined according to Sørensen (21) after the samples had been hydrolyzed as recommended by Einbinder and Schubert (22).

After dilution of the urine samples, aliquots were taken and hydrolyzed with hydrochloric acid for 30 minutes (23). At the end of hydrolysis 5 ml. of 0.05 N sodium sulfate and about 150 ml. of water were added, and the total sulfate was precipitated from the boiling solutions by the slow addition of 5 ml. of 10 per cent barium chloride. The resultant precipitate was used for assay of sulfur-35. For the determination of total sulfate-sulfur in the urine, the

TABLE I

Composition of S^{35}-Labelled Mucopolysaccharides from Various Rat Tissues at Different Ages

Source	Nitrogen	Sulfur	Uronic acid	Hexosamine
	per cent	*per cent*	*per cent*	*per cent*
S- 10	3.96	3.84	34.4	25.9
S- 30	3.28	3.82	32.5	26.1
S-300	3.25	3.48	32.1	21.4
P- 10	10.26	2.05	18.2	14.2
P- 30	4.93	3.54	31.4	29.1
P-300	3.25	4.73	42.1	42.7
V- 10	6.57	1.65	14.6	10.9
V- 30	5.50	0.99	8.7	15.7
V-300	3.37	1.55	13.8	8.6
Chondroitin-SO_4	2.69	6.15	37.3	34.4

The letters S, P, and V in column 1 indicate that the samples were isolated from skeletons, pelts, and viscera, respectively; the number after the letter indicates the age in days of the rats from whose tissues the samples were isolated.

Each value is the mean value of four separate determinations, each on a different sample.

The calculated values are for sodium chondroitin sulfate with the repeating unit weighing 520.

same procedure was followed except that the samples of urine were not diluted and no carrier sulfate was added. The barium sulfate precipitates were isolated and weighed in tared Gooch crucibles.

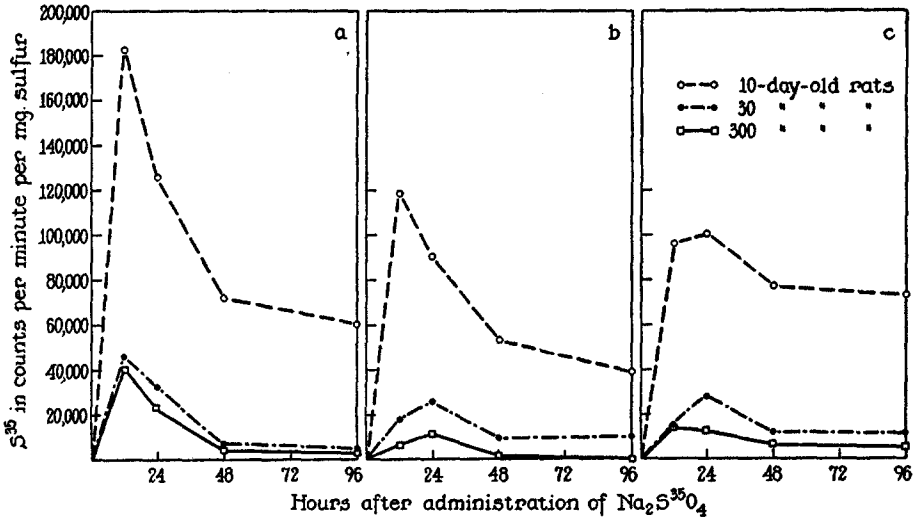
An aliquot of each serum was mixed with 5 ml. of 0.05 N sodium sulfate and oxidized with 5 ml. of Denis' reagent (18). Sulfur-35 was measured as described for urine. Inorganic sulfate-sulfur was determined by an adaptation of the method of Letonoff and Reinhold (24). Protein nitrogen was determined by micro Kjeldahl analysis (19).

A determination was also made of the fraction of sulfur-35 in each pool of sera which could pass through a cellophane membrane. Duplicate 1 ml. samples of the sera were delivered into the central tubes of dialysis cells described by Hamilton and Archibald (25). Dialysis for 24 hours against 25 ml. of water was carried out in a rocking device at 0°C. Each dialysate was then quantitatively transferred to a beaker, and to it was added 5 ml. of 0.05 N sodium sulfate, 10 ml. of 2.5 N hydrochloric acid, and enough water to bring the volume to about 200

ml. The sulfate was precipitated at the boiling point by the slow addition of 5 ml. of a 10 per cent barium chloride solution.

The residues remaining in the central tubes of the dialysis cells were transferred to porcelain evaporating dishes. After the addition of 5 ml. of 0.05 N sodium sulfate, sulfur in the samples was oxidized to sulfate with the reagent of Denis (18) and precipitated with barium chloride.

The protein components of each pool of rat sera were resolved by electrophoresis on paper (26) of 0.01 ml. samples of serum. The relative concentrations of the separated components were estimated from the amount of bromphenol blue eluted from segments 0.5 cm. wide (26).



TEXT-FIG. 5. Concentration of sulfur-35 in the acid hydrolyzable sulfate derived from the mucopolysaccharides isolated from the tissues of rats at intervals of time following the intraperitoneal injection of labelled sodium sulfate. Each of the rats was given 0.3 μc. of carrier-free sulfur-35 per gm. body weight.

(*a*) Mucopolysaccharides isolated from viscera.

(*b*) Mucopolysaccharides isolated from pelts.

(*c*) Mucopolysaccharides isolated from skeletons.

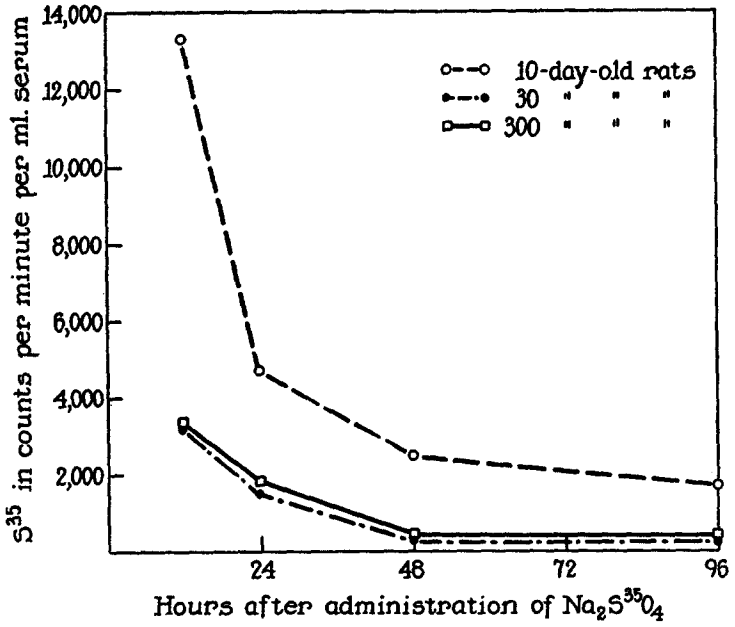
An additional sample, 2 ml. in volume, taken from the sera that had been collected 12 and 24 hours after the injection of S^{35}-labelled sodium sulfate was subjected to electrophoresis on starch (27). The starch block was cut into segments approximately 1 cm. wide; these were placed in large pyrex tubes and extracted with 2 ml. of a 1 per cent solution of sodium chloride. After removal of the starch by filtration through sintered glass, a 0.1 ml. aliquot of each filtrate was taken for the estimation of protein by a modified tyrosine method (26). Sulfur-35 in each filtrate was determined in a 1.0 ml. aliquot by delivery into a stainless steel planchet, drying under an infrared lamp, and counting in a windowless flow counter attached to a Tracerlab "1000" scaler.

RESULTS

The specific activities of sulfate-sulfur in the urines of 30- and 300-day-old rats are shown in Text-fig. 2. It can be seen that more than half of the labelled

sulfate was excreted during the first 24 hours after injection of the sodium salt. The fractions of the dose excreted at intervals of 24 hours accord with values previously recorded (2).

In Text-fig. 3 the concentration of sulfur-35 in the femoral ends is plotted against the time after administration of labelled sodium sulfate. It is seen that the concentration reached a maximum around the 24th hour in all animals and thereafter declined slowly. Although the general trends of uptake were similar,

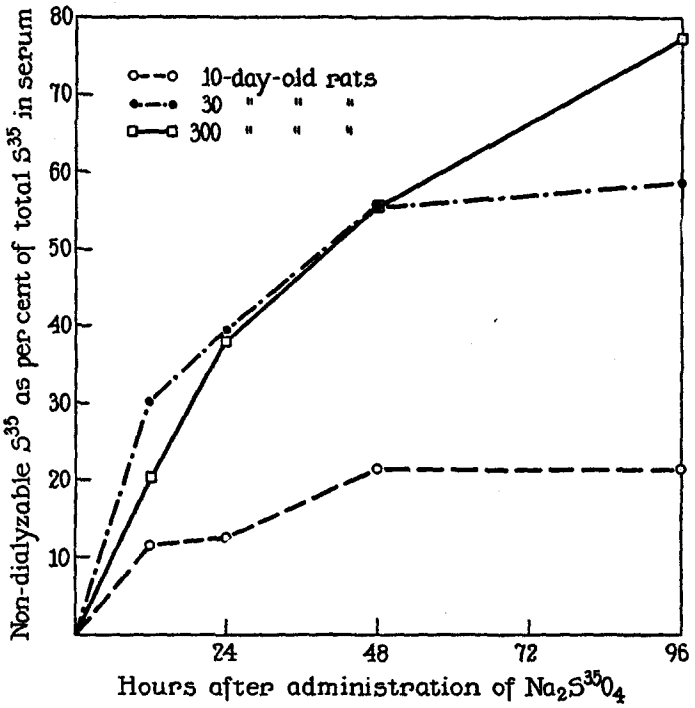


TEXT-FIG. 6. Concentration of sulfur-35 in the sera of rats at intervals of time following the intraperitoneal administration of labelled sodium sulfate. Each rat was given 0.3 μc. of carrier-free sulfur-35 per gm. body weight and the sera were collected 12, 24, 48, and 96 hours later.

there was a striking difference in the absolute concentrations from one group of animals to another: Ends of the femurs removed from the 10-day-old rats at the 24th hour after injection were found to contain 10 times and about 25 times as much sulfur-35 as the ends of femurs removed at the same time from 30- and 300-day-old rats, respectively.

The influence of age on the accumulation of sulfur-35 in the femoral shafts can be seen in Text-fig. 4. In the case of the 30-day-old rats the maximum concentration was again found at the 24th hour after sulfur-35 injection, while with the youngest and oldest rats the maximum was attained somewhat earlier. The sulfur-35 was neither as highly concentrated nor held as long in shafts as it was in the ends of the femurs.

For the most part, the above observations are in agreement with the evidence found in the radioautographs of the humeri. Figs. 1 to 12 and 13 to 20 show that the intensity of the radioautographic reaction of the humeri removed at 12 hours was nearly as strong as that of the humeri removed at 24 hours after administration of the sulfur-35. The radioautographs also indicate that the sulfur-35 did not leave the humeri rapidly. The radioautographs do more,

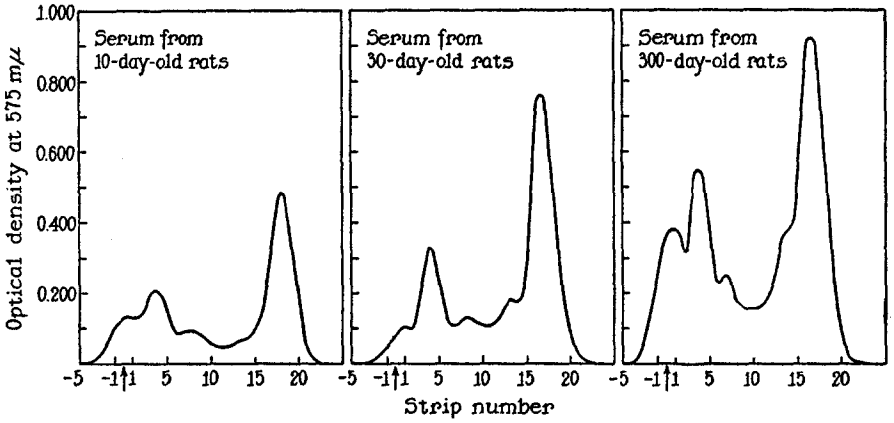


TEXT-FIG. 7. The fraction of sulfur-35 in the sera of rats, which did not pass through cellophane. Each rat was given an intraperitoneal injection of 0.3 μc. of carrier-free sulfur-35 per gm. body weight and the sera were collected 12, 24, 48, and 96 hours later.

however, than just substantiate the results of radiochemical assay; they reveal that it is in the epiphyses that the sulfur-35 is most highly concentrated and that, in the case of the 30- and 300-day-old rats, the proximal humeral epiphysis concentrated the sulfate-sulfur to a greater extent than did the distal (Figs. 5 to 8). The locations of these deposits suggest that the sulfur-35 was present for the most part in chondroitin sulfate (28).

In Figs. 13 to 20 still another aspect of the metabolism of sulfate-sulfur in humeri is apparent. Some sulfur-containing compound is deposited in the secondary centers of ossification as well as at the ends of the shaft as epiphyseal cartilage is being replaced by bone.

Data, obtained on analysis of the mucopolysaccharides isolated from the various rat tissues, are summarized in Table I. It can be seen that the composition of each of the preparations is significantly different from that of sodium chondroitin sulfate. The samples which most nearly resemble the postulated sodium chondroitin sulfate are the ones isolated from the skeletons. On the other hand, the mucopolysaccharides isolated from the pelts and viscera show a diversity of compositions far removed from the expected values. Each sample



TEXT-FIG. 8. Electrophoretic analysis of the proteins in sera of rats at different ages. The sera were resolved on filter paper and the concentrations of protein at different positions measured by binding of bromphenol blue (26). Each curve is the mean of four separate determinations made on different pools of sera. The arrows indicate the points at which the sera were applied.

(*a*) Sera from 10-day-old rats. Total protein, 3.94 gm. per cent. Inorganic sulfate-sulfur 2.24 mg. per cent.

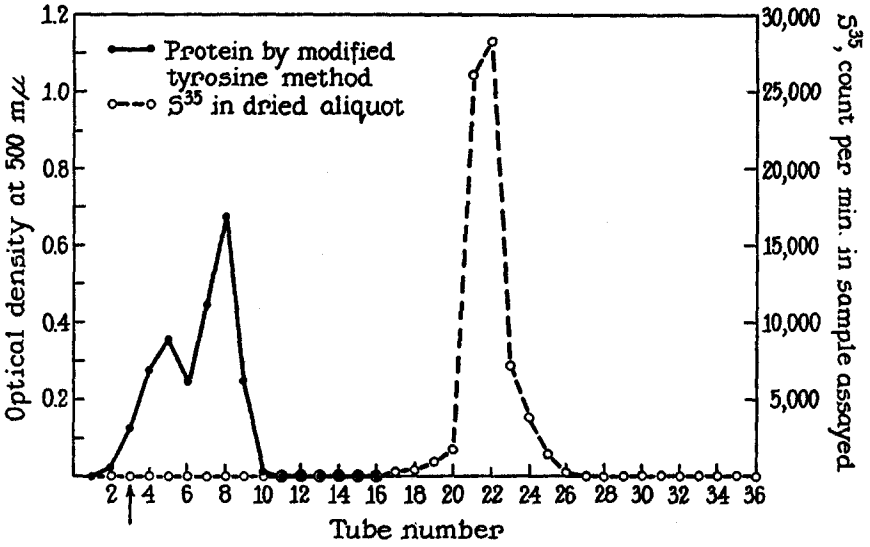
(*b*) Sera from 30-day-old rats. Total protein, 5.75 gm. per cent. Inorganic sulfate-sulfur, 2.08 mg. per cent.

(*c*) Sera from 300-day-old rats. Total protein, 6.75 gm. per cent. Inorganic sulfate-sulfur, 1.66 mg. per cent.

is undoubtedly a mixture of materials, whose nature and ratio probably vary from one age group to another as well as from one tissue to another.

Despite these differences of composition one can compare the concentrations of sulfur-35 when the results are calculated in terms of specific activity (*viz.*, counts per minute per milligram of acid hydrolyzable sulfate-sulfur). The curves in Text-fig. 5 *a* show that the specific activity of sulfur-35 is highest in the mucopolysaccharides isolated from the viscera of the rats 12 hours after injection of the isotope. In the samples from the rats sacrificed 96 hours after administration of the isotope the values were 33.2, 11.5, and 8.7 per cent of the maximum values observed in the samples from the 10-, 30-, and 300-day-old rats, respectively.

From the data presented in Text-fig. 5 *b* it can be seen that the mucopolysaccharides isolated from the pelts of 10-day-old rats showed a peak of specific activity 12 hours after administration of labelled sodium sulfate. In the 30- and 300-day-old rats the peak was found at 24 hours after injection. At the 96th hour the values for specific activity were 33.4, 42.1, and 12.8 per cent of the maximum values observed for samples from the 10-, 30-, and 300-day-old rats, respectively.

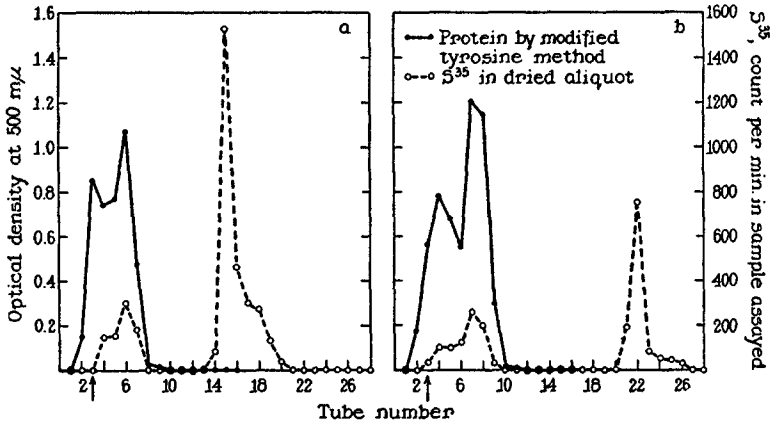


TEXT-FIG. 9. Electrophoretic separation of inorganic sulfate from the proteins of rat serum. Carrier-free sulfur-35 as 0.05 ml. of sodium sulfate solution was added to 2 ml. of adult rat serum. 1 ml. of the tagged serum was diluted with 1 ml. of barbital buffer, pH 8.6, μ 0.1, and the diluted serum was then placed on a starch block. The electrophoretic separation was produced under the influence of a potential difference of 600 volts, 30 ma., in 7 hours at 0°C. The protein and sulfur-35 contents in approximately 1 cm. wide segments of the starch block were determined as detailed in the text, except that each segment was mixed with 4 instead of 2 ml. of a 1 per cent sodium chloride solution.

In Text-fig. 5 *c* it can be seen that the highest concentration of sulfur-35 in the preparations of chondroitin sulfate from the skeletons of the 10- and 30-day-old rats was observed 24 hours after administration of the S^{35}-labelled sodium sulfate. The concentration of the isotope was higher in the 12 hour sample than in the 24 hour sample isolated from the skeletons of the 300-day-old rats. In each age group, up to the 96th hour after injection, the concentration of sulfur-35 in the skeletal mucopolysaccharides remained higher than that in the mucopolysaccharides isolated from the viscera or skins. The skeletal samples at the 96th hour had specific activities which were 72.4, 45.1, and 41.4 per cent of the maximum values found in the samples from the 10-, 30-, and 300-day-old rats, respectively.

In all three tissues it was found that the concentration of sulfur-35 in the samples isolated from 10-day-old rats was strikingly higher than that in corresponding samples from 30-day-old rats. Likewise, the samples from 30-day-old rats had a higher concentration of sulfur-35 than those from 300-day-old rats.

It was expected that the administration of the same quantity of sulfur-35 per gram body weight would result in comparable levels of sulfur-35 in the blood of all the animals. This expectation was realized in the case of the 30- and 300-day-old rats (Text-fig. 6), but it was a surprise to find that the concentra-



TEXT-FIG. 10. Electrophoretic patterns of sulfur-35–containing components in adult rat sera following intraperitoneal injection of labelled sodium sulfate. The separation was effected in barbital buffer, pH 8.6, μ 0.1, in a starch block at 600 volts and 30 ma. at 0°C. 2 ml. of serum mixed with 0.5 ml. of the barbital buffer was used. The protein and sulfur-35 content of 1 cm. wide segments were estimated as detailed in the text.

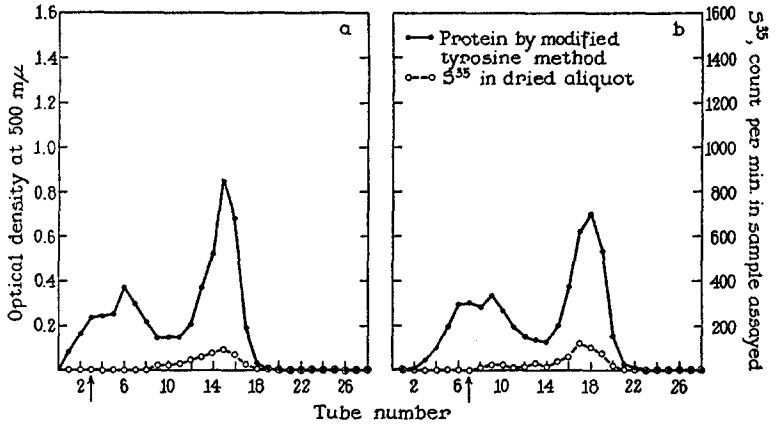
(*a*) Serum collected 12 hours after administration of S^{35}-labelled sodium sulfate. Resolution was allowed to proceed for 6.5 hours.

(*b*) Serum collected 24 hours after administration of S^{35}-labelled sodium sulfate. Resolution was allowed to proceed for 7.25 hours.

tion of sulfur-35 in the sera of 10-day-old rats was 3 to 5 times as high as that in the sera of 30- and 300-day-old rats. In an investigation of this difference in sulfur-35 levels, the sera were dialyzed against water. It was found (Text-fig. 7) that a smaller fraction of the sulfur-35 in the sera of the 10-day-old animals was retained by cellophane than was the case with the sera of the older animals. This fraction of sulfur-35 in the sera of the older rats continued to increase with time after injection, whereas the non-dialyzable fraction in the sera of 10-day-old rats remained constant from the 48th to the 96th hour.

Since a binding of various ions to proteins is well known (29) and an age difference in blood protein levels has been reported (30), the sera of the different age groups were subjected to electrophoretic analysis on paper and on starch. In addition, the inorganic sulfate-sulfur and protein (based on nitrogen) con-

centrations in the sera were determined. It was found that the 10-day-old rats had the highest concentrations of inorganic sulfate in their sera, and the lowest concentrations of protein (Text-fig. 8). In each of the averaged patterns of Text-fig. 8, five components can be made out. In general the concentrations of individual components increased with age; the one exception noted was a somewhat greater concentration of gamma globulin in the 10-day-old rats than in the 30-day-old group.



TEXT-FIG. 11. Electrophoretic patterns of sulfur-35 containing components in adult rat sera following intraperitoneal injection of labelled sodium sulfate. For further details consult legend to Text-fig. 10.

(*a*) Serum collected 12 hours after administration of the isotope. This is the same serum shown in Text-fig. 10 *a*, except that resolution of its components was allowed to proceed for 24.5 hours.

(*b*) Serum collected 24 hours after administration of sulfur-35. This is the same serum shown in Text-fig. 10 *b*, except that resolution of its components was allowed to proceed for 24.5 hours.

Adult rat sera with carrier-free sulfur-35 (as sodium sulfate) added *in vitro* were subjected to electrophoresis on starch blocks according to the technique described by Kunkel and Slater (27). All of the added inorganic sulfate was readily separated from the proteins (Text-fig. 9). No sulfur-35 could be detected in the protein bands even when the mixture of serum and labelled sulfate had stood for 3 months at 0°C. before analysis. In sharp contrast, after administration of S^{35}-labelled sodium sulfate to rats, some of the sulfur-35 rapidly became associated with proteins. In Text-fig. 10 the electrophoretic patterns for protein and sulfur-35 are shown for sera from adult rats sacrificed 12 and 24 hours after injection of sulfur-35. It can be seen that in each case two peaks of sulfur-35 could be distinguished. The faster moving one of these peaks probably was mainly inorganic sulfate, while the slower coincided with the faster moving components of protein. Experiments done with 10- and 30-day-

old rats gave similar results. In an attempt to ascertain whether the sulfur-35 in the slower moving peak was associated with some of the globulins as well as with albumin, electrophoresis of other sera was extended for approximately 24 hours. The resultant patterns, as shown in Text-fig. 11, indicate that the sulfur-35 was associated not only with the albumin fraction but with the globulins too. The distributions of protein-bound sulfate are to some extent reminiscent of the patterns reported for iodine-131 in sera following administration of I^{131}-labelled sodium iodide (31).

DISCUSSION

From studies on the uptake of sulfate-sulfur by various animal tissues *in vitro* (13, 14) and *in vivo* (15) one would expect age to influence the turnover rate of sulfate-sulfur in mucopolysaccharides. This expectation was realized in the study reported here. The mucopolysaccharides of the tissues in the youngest rats were expected to accumulate the largest amount of the sulfur-35, and indeed, they did, (Text-fig. 5). It was not expected, however, to find that the specific activity of the mucopolysaccharides from the tissues of the 10-day-old rats would decrease at a slower rate than the rate of decrease shown by the mucopolysaccharides from the tissues of the 30- and 300-day-old rats. Yet they did, in all instances but one. The single exception was a somewhat more rapid decrease of specific activity in the case of mucopolysaccharides extracted from the pelts of 10-day-old rats, as compared with similar preparations from 30-day-old rats.

The values for sulfur-35 concentration in the ends and shafts of the femurs from the rats of different ages supported the assumption that the isotope had been incorporated into chondroitin sulfate. The concentration of sulfur-35 was found to be highest in those regions which contained the most cartilage.

The radioautographs of the humeri fixed in formalin and in formalin saturated with barium hydroxide confirmed and supplemented the analytical data. In addition to localizing the greatest accumulation of sulfur-35 in the epiphyseal cartilage, the radioautographs showed that different regions of the epiphyseal cartilage incorporate the isotope at different rates. The observation that the proximal epiphysis of the humerus in the 30-day-old rat is more active than the distal is in agreement with the conclusions reached by others on the basis of histologic studies. It has been reported that the distal epiphysis of the rat humerus shows closure by the 40th day of age, whereas the proximal epiphysis of this bone is open up to about the 1200th day of life. (32).

If one compared only the data on the 10-day-old rats with the data on either the 30- or the 300-day-old rats one would not be justified in unreservedly concluding that there was a direct relationship between age and rate of sulfate-sulfur metabolism in the mucopolysaccharides. One could be equally convinced that the observed differences in the level of sulfur-35 in the mucopolysaccharides

were attributable to the striking differences in the levels of sulfur-35 in the sera of the 10-day-old animals as compared with those 30 or 300 days old even though, per unit weight, each animal received the same amount of labelled sodium sulfate. One might argue that the observed differences in the sulfur-35 levels in the mucopolysaccharides bore a direct relationship to the serum levels of sulfur-35, which were different because of the influence of age on some factor affecting the clearance of sulfate-sulfur from the blood. It cannot be denied that the observed higher level of sulfur-35 in the sera of the youngest animals may be reflected in the strikingly higher concentration of sulfur-35 in their mucopolysaccharides. It is our belief, however, that the age difference among the animals is also directly reflected in the sulfur-35 levels of the mucopolysaccharides. It is concluded that the rate of sulfate-sulfur metabolism in the mucopolysaccharides of the youngest animals is more rapid than that in the older animals. This conclusion seems unescapable from an examination of the data on the 30-day-old and the 300-day-old rats. In Text-fig. 6 it can be seen that the sulfur-35 levels in the sera of the 30-day-old rats were close to but still consistently lower than the levels of the isotope in the sera of the 300-day-old rats. Yet, despite this approximation in the serum levels, the levels of sulfur-35 in the mucopolysaccharides from the tissues of the 30-day-old rats were significantly higher than those of the mucopolysaccharides from the 300-day-old rats.

Further assurance that there is a direct relationship between the age of the animal and the rate at which sulfate-sulfur is incorporated into the mucopolysaccharides comes from a recent report by Boström and Månsson (33). It was observed by them that slices of tracheal cartilage from a suckling calf, incubated *in vitro* with labelled sulfate, were about twice as active as those from a 2 year old heifer, and these in turn were about twice as active as the slices from the trachea of a 12 year old cow.

Although a reason for the difference in the sulfur-35 levels of the sera from the 10-day-old and older rats was sought no explanation is as yet available. In the course of the search, however, it was found that the fraction of sulfur-35 in the sera which is bound to protein is smaller in younger than in older rats. This difference in binding of administered sulfate-sulfur is perhaps a result, to some extent at least, of the difference in serum protein level with age (Text-fig. 8). Electrophoresis of the rat sera confirmed the results obtained in the dialysis experiments in regard to the binding of sulfur-35 initially administered as sulfate-sulfur. The values for the fraction of sulfur-35 which was bound were essentially the same whether dialysis or electrophoresis was used. The electrophoretic studies also indicate that very little, if any, sulfate is bound to protein if it is added *in vitro*, (Text-fig. 9). On the other hand, sulfate-sulfur when ingested somehow becomes associated with serum proteins, mainly with albumin, and to a lesser extent with α- and β-globulins.

SUMMARY

S^{35}-labelled sodium sulfate was administered to rats 10, 30, and 300 days old in an intraperitoneal dose of 0.3 μc. per gm. of body weight. Representative animals of each age were sacrificed 12, 24, 48, and 96 hours after injection.

The concentration of sulfur-35 in the pooled sera of the 10-day-old rats was found to be strikingly higher than the level in the sera of the 30-day-old and the 300-day-old rats, while the levels of sulfur-35 in the sera of rats in the latter two age groups were similar. The difference was not explained by the differences in binding of sulfate by serum proteins. Although no binding could be detected when sulfate was added to serum *in vitro*, a substantial fraction, up to 80 per cent by the 96th hour, was observed to be bound after injection into the living rat. The 10-day-old rats differed from the older ones in having lower levels of serum proteins and lesser amounts of bound sulfate. The nondialyzable sulfur-35 was associated to the largest extent with the albumin component in the sera.

The age of the rats found expression in the specific activities of the sulfate-sulfur of mucopolysaccharides isolated from the skeletons, pelts, and viscera. The highest specific activities were observed in the mucopolysaccharides isolated from the tissues of the youngest rats; the lowest in those from the oldest rats. Though the maximum concentration was rapidly attained in the mucopolysaccharides from the various tissues in each of the age groups, the subsequent decreases in concentration were slow.

Radiochemical analyses for sulfur-35 in ends and shafts of femurs and radioautographs of humeri supported the assumption that the labelled sulfate had been incorporated into the chondroitin sulfate of growing cartilage.

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EXPLANATION OF PLATES

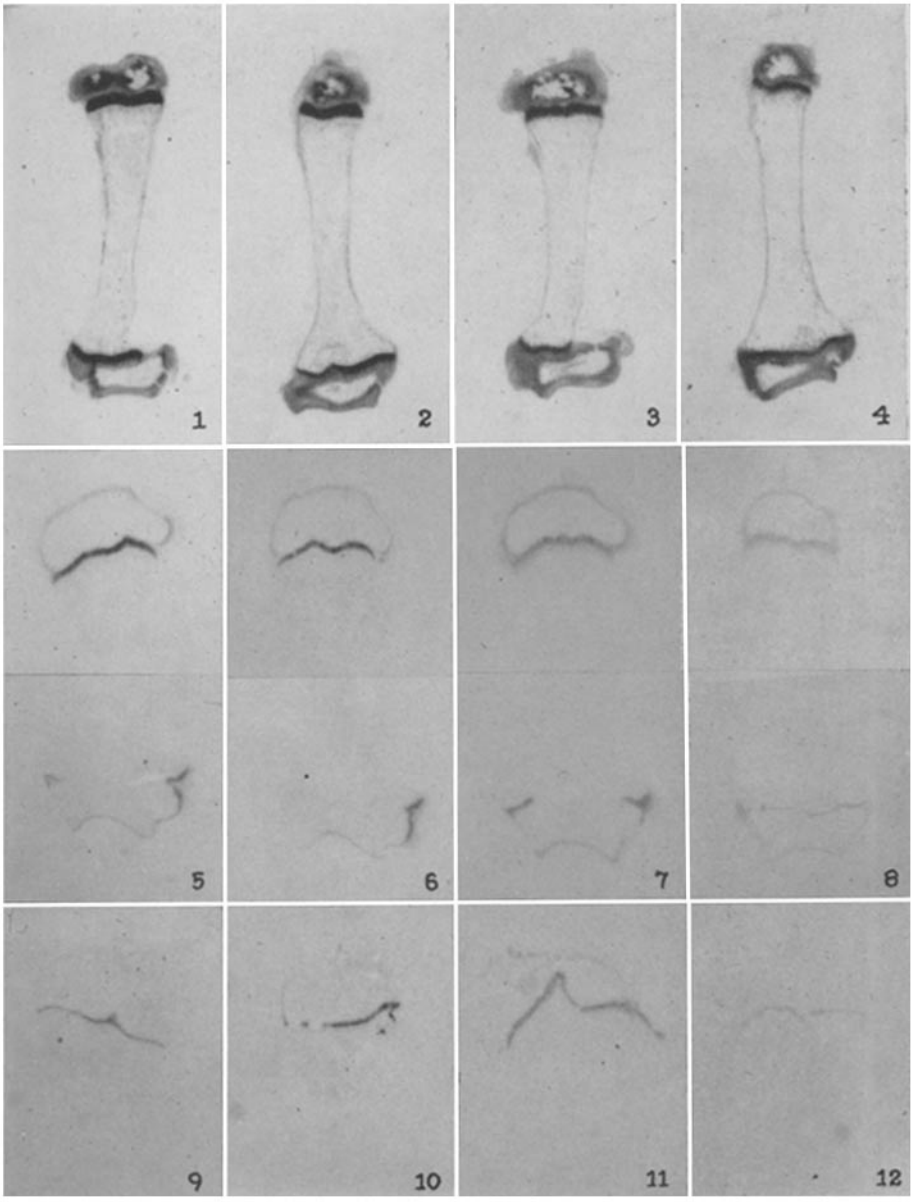
PLATE 6

Radioautographs produced by sections of humeri fixed in a 3.7 per cent solution of formaldehyde for 48 hours at 25°C. Kodak contrast process ortho film was exposed to the sections for 4 weeks at 20°C. ×3.9.

FIGS. 1 to 4. Photographs of radioautographs produced by sections of humeri removed from 10-day-old rats 12, 24, 48, and 96 hours, respectively, after the intraperitoneal administration of 0.3 μc. of sulfur-35 as sodium sulfate per gm. body weight.

FIGS. 5 to 8. Photographs of radioautographs produced by sections of humeri removed from 30-day-old rats 12, 24, 48, and 96 hours, respectively, after the intraperitoneal administration of 0.3 μc. of sulfur-35 as sodium sulfate per gm. body weight. The large size of the humerus in 30-day-old rats prevented section of the whole bone, as was possible with the 10-day-old rat; therefore only the ends are shown.

FIGS. 9 to 12. Photographs of radioautographs produced by sections of the proximal ends of the humeri removed from 300-day-old rats 12, 24, 38, and 96 hours, respectively, after the intraperitoneal administration of 0.3 μc. of sulfur-35 as sodium sulfate per gm. body weight. The distal epiphyses of the 300-day-old rats' humeri had a barely discernible effect on the photographic film and so are not included.



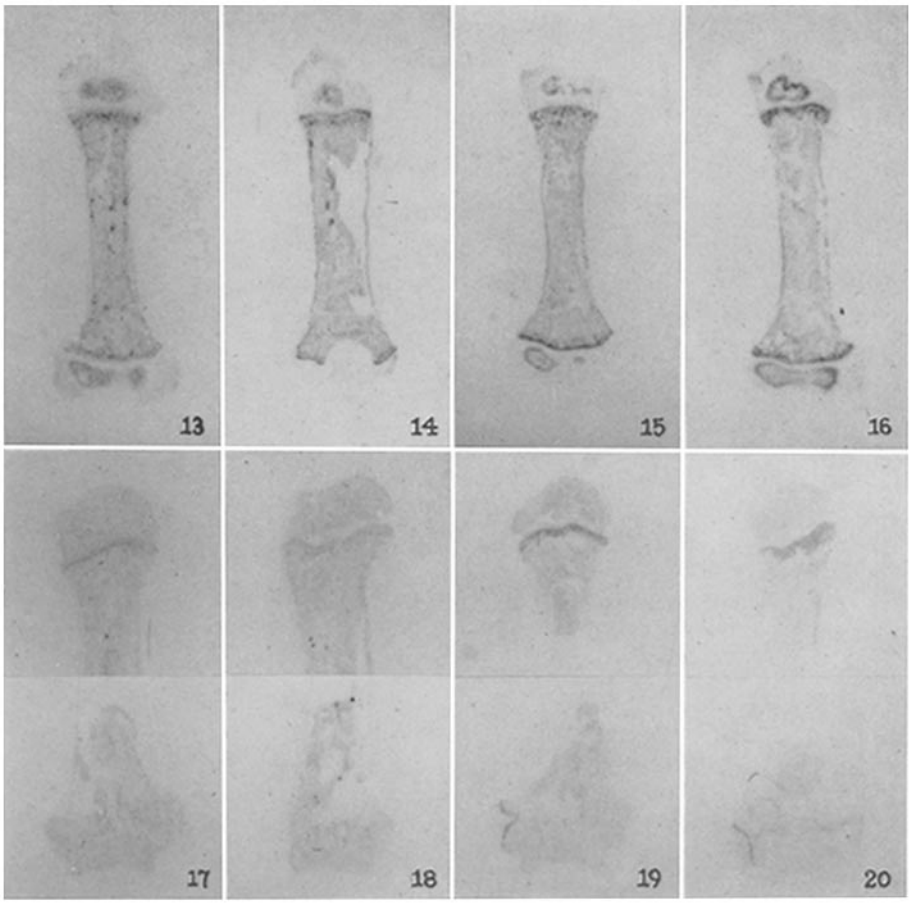
(Dziewiatkowski: Effect of age on sulfate metabolism in the rat)

PLATE 7

Radioautographs produced by sections of humeri fixed for 48 hours at 25°C. in a 3.7 per cent solution of formaldehyde previously saturated with $Ba(OH)_2$. Kodak contrast process ortho film was exposed to the sections for 17 weeks at 20°C. ×3.9.

FIGS. 13 to 16. Photographs of radioautographs produced by sections of humeri removed from 10-day-old rats 12, 24, 48, and 96 hours, respectively, after the intraperitoneal administration of 0.3 μc. of sulfur-35 per gm. body weight.

FIGS. 17 to 20. Photographs of radioautographs produced by sections of humeri removed from 30-day-old rats 12, 24, 48, and 96 hours, respectively, after the intraperitoneal administration of 0.3 μc. of sulfur-35 as sodium sulfate per gm. body weight.



(Dziewiatkowski: Effect of age on sulfate metabolism in the rat)