SYNTHESIS OF SULFOMUCOPOLYSACCHARIDES IN THYROIDECTOMIZED RATS

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PLATES 5 TO 8

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Thiouracil depresses and thyroxine accelerates the disappearance of $^{35}$S-sulfate from the knee joints of suckling rats (1). Apparently thyroxine affected the catabolism of chondroitin sulfate, since nearly all of the $^{35}$S-sulfate in the cartilage was present as chondroitin sulfate (2, 3).

The purpose of the present experiments was to determine whether thyroxine has a specific effect on the synthesis of chondroitin sulfate. It is well known that an insufficient secretion of the thyroid hormone in young animals results in a generalized retardation of growth and development. Presumably the synthesis of chondroitin sulfate is depressed. However, experimental cretinism in rats produced by surgical removal (4, 5) is a mixed syndrome: the parathyroids so indent the thyroid that they are also removed. One cannot with certainty ascribe observed metabolic changes to lack of the thyroid hormone. Although replacement therapy with parathyroid extract is possible, obviously it would be preferable to retain the parathyroids. This can be accomplished by thyroidectomy with radioactive iodine (iodine-131), as developed by Goldberg and Chaikoff (6). They found that 80 to 150 $\mu$c. of iodine-131 injected into a rat several hours after birth completely destroyed the thyroid gland. The parathyroids, on the other hand, except for a thickened capsule, a few pyknotic cells in the peripheral portions, and some increase in interstitial connective tissue, appeared essentially normal histologically. Both methods of thyroidectomy, surgical and pharmacological, were used in the present studies. The amount of $^{35}$S-sulfate which the thyroidectomized rats utilized in the synthesis of sulfomucopolysaccharides of the skeleton and of the skin was then compared with the amount utilized by intact rats of the same age.

Procedure

Carrier-free iodine-131 as sodium iodide in water was injected intraperitoneally into each of thirty 1-day-old rats of the Sherman strain. The dose was 100 $\mu$c. per rat. After 28 days with their respective mothers, 16 of the rats were still alive. They were divided into two

1 The iodine-131 and sulfur-35 were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission.
groups and were then fed a diet low in iodine, Remington's diet 342 (7). In addition, each rat in one of the groups was given 5 $\gamma$ of $l$-thyroxine daily by intraperitoneal injection. Twenty normal rats of the same age served as controls; ten of these were fed the low iodine diet, while the remaining ten rats were maintained on Purina fox chow. Fourteen days later, on the 42nd day of life, carrier-free sulfur-35 as sodium sulfate in water was injected intraperitoneally; 1 $\mu$g per gm. of body weight was used. The rats were sacrificed 12 hours later by exsanguination under deep ether anesthesia.

A humerus, the proximal end of a tibia, and a ring of tail skin were removed from each animal and fixed in 10 per cent formalin for 48 hours. After dehydration in alcohol, roentgenograms of the humeri were taken. The tibiae and tail skins were embedded in paraffin, sections of 7 $\mu$m were cut, and these were then used to prepare contact autoradiograms (8). Subsequently, the sections were stained with 0.1 per cent toluidine blue in 30 per cent ethanol.

The right femur was removed from each animal for the determination of the concentration of sulfur-35 in the ends and shaft (9). The concentration of the isotope in pooled sera was similarly determined (9).

After removing most of the musculature, the remainders of the skeletons from the animals in each group were separately pooled. Similar pools were made of the pelts. Mucopolysaccharides were isolated from the pooled tissues and then analyzed as previously described (9).

In the second series of experiments the thyroids were removed surgically from thirty 28-day-old rats. The technique of thyroid removal and the subsequent care of these animals

TEXT-FIG. 1. Growth of rats thyroidectomized by the use of iodine-131. To destroy the thyroids, each rat was given intraperitoneally 100 $\mu$m of iodine-131 on the 1st day of life. Except for the rats in one control group, as indicated, all the rats were fed a diet low in iodine after weaning on the 28th day of life. Thyroxine was administered to the animals in one group; 5 $\gamma$ was injected daily into each rat from the 28th to the 42nd day of life. The plotted values are average weights of the rats, the number of which in each group is shown to the right of a curve.
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TABLE I

Weight Gain of Surgically Thyroidectomized Rats

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>28th day gm.</th>
<th>42nd day gm.</th>
<th>Gain gt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>II A</td>
<td>Without thyroxine</td>
<td>59.0</td>
<td>78.8</td>
<td>19.8</td>
</tr>
<tr>
<td>II B</td>
<td>5 γ thyroxine daily</td>
<td>54.0</td>
<td>80.4</td>
<td>26.4</td>
</tr>
<tr>
<td>II C</td>
<td>10 γ thyroxine daily</td>
<td>57.0</td>
<td>89.6</td>
<td>32.6</td>
</tr>
<tr>
<td>II D</td>
<td>Mock operation, low iodine</td>
<td>56.0</td>
<td>93.2</td>
<td>37.2</td>
</tr>
<tr>
<td>II E</td>
<td>Controls, stock diet</td>
<td>52.0</td>
<td>93.8</td>
<td>41.8</td>
</tr>
</tbody>
</table>

The thyroids were surgically removed from the rats on their 28th day of life. Thyroxine when injected was given intraperitoneally daily for the following 14 days. There were 10 rats in each group.

TABLE II

Concentration of Sulfur-85 in Sera and Femurs, and in the Sulfomucopolysaccharides of Skeletons and Pelts of Thyroidectomized Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum C.P.M./ml.</th>
<th>Femurs Ends C.P.M./mg.</th>
<th>Femurs Shafts C.P.M./mg.</th>
<th>Skeleton C.P.M./mg.</th>
<th>Pelt C.P.M./mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment I, thyroidectomy with I-131</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>Without thyroxine</td>
<td>7,250</td>
<td>13.4</td>
<td>12.6</td>
<td>9,275</td>
<td>4,950</td>
</tr>
<tr>
<td>IB</td>
<td>5 γ thyroxine daily</td>
<td>1,050</td>
<td>12.0</td>
<td>5.4</td>
<td>15,840</td>
<td>8,700</td>
</tr>
<tr>
<td>IC</td>
<td>Controls, low iodine</td>
<td>2,765</td>
<td>20.4</td>
<td>9.2</td>
<td>31,130</td>
<td>29,500</td>
</tr>
<tr>
<td>ID</td>
<td>Controls, stock diet</td>
<td>975</td>
<td>11.3</td>
<td>3.9</td>
<td>18,990</td>
<td>26,800</td>
</tr>
<tr>
<td></td>
<td>Experiment II, surgical thyroidectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>Without thyroxine</td>
<td>3,110</td>
<td>16.7</td>
<td>8.5</td>
<td>21,250</td>
<td>20,300</td>
</tr>
<tr>
<td>IB</td>
<td>5 γ thyroxine daily</td>
<td>3,030</td>
<td>16.1</td>
<td>6.9</td>
<td>40,120</td>
<td>29,000</td>
</tr>
<tr>
<td>IC</td>
<td>10 γ thyroxine daily</td>
<td>2,675</td>
<td>15.7</td>
<td>6.6</td>
<td>27,210</td>
<td>25,400</td>
</tr>
<tr>
<td>ID</td>
<td>Controls, low iodine</td>
<td>2,690</td>
<td>20.3</td>
<td>8.8</td>
<td>35,960</td>
<td>28,500</td>
</tr>
<tr>
<td>IE</td>
<td>Controls, stock diet</td>
<td>1,160</td>
<td>13.8</td>
<td>4.7</td>
<td>22,200</td>
<td>26,500</td>
</tr>
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</table>

In sodium chondroitin sulfate one expects to find 2.69 per cent nitrogen, 6.15 per cent sulfate-sulfur, 37.3 per cent glucuronic acid, and 34.4 per cent hexosamine. In the preparations which were isolated from the pelts the ranges of values were: nitrogen, 4.2 to 8.6; glucuronic acid, 14.4 to 16.8; hexosamine, 6.5 to 9.3; sulfate-sulfur, 1.6 to 2.3. The materials isolated from the skeletons had 4.3 to 4.9 per cent nitrogen; 14.5 to 18.1 per cent glucuronic acid; 13.8 to 16.3 per cent hexosamine; and 2.6 to 3.9 per cent sulfate-sulfur.

were those described by Leblond and Earley (5). The rats were divided into three groups of ten animals each and fed Remington's diet 342 (7). In addition, each animal in one of the groups was given 5 γ of L-thyroxine daily by intraperitoneal injection; each animal in a second group was given similarly 10 γ of L-thyroxine daily. Two additional groups of ten
animals each served as controls. The animals in one of these groups were subjected to a mock thyroidec
tomy on their 28th day of life and were then fed Remington's diet 342. The rats in
the other control group were fed Purina fox chow from the 28th day of life until the experi-
ment was terminated.

The animals in the second set of experiments were also sacrificed on their 42nd day of
life, 12 hours after having received an intraperitoneal injection of sulfur-35 given in a dose
of $1\,\mu$C per gm. of body weight. The carrier-free isotope was administered as sodium sulfate
in water. The tissues were analyzed as were those from the rats in the first series of experi-
ments.

Examination of serial sections of the neck region of representative thyroidec
tomized animals from each of the two series of experiments revealed that thyroid tissue was absent
from the neck region. Parathyroid tissue was found in the animals that had received iodine-
131. It was essentially normal in appearance and amount but was surrounded by collagenous
tissue.

RESULTS

The rats that were thyroidec
tomized with iodine-131 did not grow as rapidly
as the control animals (Text-fig. 1). Thyroxine administered in a dose of $5\,\gamma$
daily for 14 days stimulated growth and restored the alertness of the rats
(Plate 5). Though the change in appearance of the surgically thyroidec
tomized rats was less striking, they also grew more slowly than normal rats or thyroidec
tomized rats given supplements of thyroxine (Table I).

No change in the humeri of the surgically thyroidec
tomized rats was seen
in the roentgenograms. On the other hand, the humeri of rats thyroidec
tomized with iodine-131 failed to grow and ossify to the same extent as those of the
control or the thyroidec
tomized rats given $5\,\gamma$ of thyroxine daily for the
previous 2 weeks (Plate 6).

The concentrations of sulfur-35 in the sera and femurs, and in the sulfo-
mucopolysaccharides isolated from the skeletons and pelts of the animals in
the two series of experiments, are given in Table II. Although the concentra-
tion of sulfur-35 in the sera of the animals thyroidec
tomized with iodine-131
was much higher than in the sera in the other groups, the uptake of sulfur-35
into the bones was no greater, except in the femoral shafts. The concentra-
tion of sulfur-35 in the ends of the femurs removed from the thyroidec
tomized animals was approximately the same as in the femoral ends of animals in
groups I B and I D, and was lower than that found in group I C. The specific
activity of the sulfate-sulfur in the sulfomucopolysaccharides isolated from the
skeletons and pelts of the untreated thyroidec
tomized rats, however, (group
I A) was markedly lower than the activity of comparable material isolated
from control rats or thyroidec
tomized animals treated with $5\,\gamma$ of thyroxine
daily, Table II.

Surgical thyroidec
tomy (groups II A to II E in Table II) likewise depressed
the uptake of sulfur-35, although to a lesser degree.

The autoradiograms reproduced in Plates 7 and 8 support the analytical
data. In Plate 7, it can be seen that the uptake of sulfur-35 into the epiphysial cartilage plate region of the proximal end of a tibia from an untreated thyroidectomized rat (Fig. 1) was very weak indeed compared with the uptake into the tibia of a thyroidectomized rat given 5 \( \gamma \) of thyroxine daily (Fig. 2) or the tibiae of the control animals (Figs. 3, 4). A similar difference can be seen in the autoradiograms of skin sections; for example, compare Fig. 9 with Figs. 10, 11, 12. The autoradiograms of tibiae from the animals in the second series of experiments, Plate 8, show a similar, but less marked, depression of uptake by the surgically thyroidectomized rats (compare Fig. 13 with Figs. 14, 15, 16, and 17).

**DISCUSSION**

For 12 hours and more after the administration of \( {^{35}}S \)-sulfate to rats its concentration in the sulfomucopolysaccharides of the skeleton and pelts continues to increase (3, 9, 10): the synthetic reaction is dominant. Hence the data in the last two columns of Table II suggest that the synthesis of sulfomucopolysaccharides in the skeletons and pelts was impaired by thyroidectomy. Parenterally administered thyroxine in some way restored or prevented the deterioration of the synthetic mechanisms.

Further support for this interpretation was given by the autoradiograms of tibiae and pelts. The sections were cut from tissue blocks which had been fixed in aqueous, slightly acidic formalin. In the process of fixation inorganic sulfate was undoubtedly removed, leaving behind \( {^{35}}S \)-sulfate in the sulfomucopolysaccharides.

In designing the experiments it was thought that the total concentration of sulfur-35 in the tissues would reflect the specific activity of the sulfate in the sulfomucopolysaccharides. Under the experimental conditions used, this did not prove to be true. For example, despite the fact that the concentration of sulfur-35 in the ends of femurs from the thyroidectomized rats was in the same range as the concentration in the ends of femurs from similarly prepared but thyroxine-supplemented rats, compare group I A with group I B, and group II A with groups II B and II C, the specific activities of the sulfomucopolysaccharides isolated from the skeletons of the former were significantly lower than the specific activities of the materials isolated from the skeletons of the latter. One does see that the expected correlation is partially realized only when the unsupplemented thyroidectomized rats are compared with the intact rats on the low iodine diet.

It is not surprising that the effects of thyroidectomy by the use of iodine-131 were more striking than the effects of surgical removal of the thyroids; iodine-131 was administered to the rats on their 1st day of life whereas surgical thyroidectomy was deferred to the 28th day. Definite signs of hypothyroidism were noted in the iodine-131 rats by the end of the 2nd week. The quantitative
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differences presumably reflect differences in the duration of hypothyroidism. Qualitatively, the results were similar.

SUMMARY

The thyroid glands of rats were made non-functional by a single dose of iodine-131 on the 1st day after birth or by surgical removal on the 28th day. The incorporation of sulfate-sulfur into the sulfomucopolysaccharides of skeletons and pelts was found to be significantly depressed by thyroidectomy. Daily supplements of 5 or 10 $\gamma$ of L-thyroxine, started on the 28th day, increased the uptake of sulfur-35, although it did not reach normal in the 2 weeks of treatment.

Autoradiograms of sections of tibiae and pelts confirmed the analytical data. The findings suggest that the synthesis of sulfomucopolysaccharides is depressed in thyroidectomized rats.

BIBLIOGRAPHY


EXPLANATION OF PLATES

PLATE 5

Photographs of representative thyroidectomized and normal rats on their 42nd day of life. The thyroidectomized rats had their thyroids destroyed by 100 $\mu$g of iodine-131, given intraperitoneally on the 1st day of life. As a result, the rats did not grow normally. They were sluggish and at the end of 42 days of life sat as shown in the upper photograph. Daily administration of 5 $\mu$g of L-thyroxine from the 28th day to the 42nd day of life made them more alert, as can be seen in the middle photograph. For the last 2 weeks of the experiment the thyroidectomized rats and thyroxine-supplemented thyroidectomized rats were fed a diet low in iodine. The normal rat shown was fed a stock diet. $\times 0.25.$

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Thyroidectomized rat
42nd day of life.

5 γ thyroxine daily for 14 days.

Normal rat,
42nd day of life.

(Dziewiatkowski: Sulfomucopolysaccharides in thyroidectomized rats)
PLATE 6

Roentgenogram of humeri removed from rats on the 42nd day of life. Each humerus is from a different rat. The humeri were fixed in 10 per cent aqueous formalin and dehydrated in alcohol prior to radiography. It can be seen that the bones of the rats thyroidectomized by the administration of 100 #µc. of iodine-131 on their 1st day of life, group I A, are smaller and less well calcified. They are immature compared with the bones from similar rats treated daily with 5 γ of L-thyroxine, group I B, from the 28th day to the 42nd day of life. In group I C are the humeri from intact rats fed the same low-iodine diet as was fed to the rats the bones of which are shown in groups I A and I B. Humeri from control rats on a stock diet are shown in group I D. × 1.
(Dziewiatkowski: Sulfomucopolysaccharides in thyroidectomized rats)
Effect of thyroidectomy with iodine-131 on the uptake of S\textsuperscript{35}-sulfate by tibiae and skin. Autoradiograms produced by sections of the proximal ends of tibiae and tail skins from 42-day-old rats. The tissues were fixed in a 10 per cent solution of formalin for 48 hours at 25°C. Kodak contrast process ortho film was exposed to the sections for 4 weeks. Each animal received per gm. of body weight 1 μc. of sulfur-35 as sodium sulfate 12 hours before sacrifice. × 5.

Directly to the right of an autoradiogram of a tibia is a photograph of the stained section which produced that autoradiogram. The sections were stained with 0.1 per cent toluidine blue in 30 per cent ethanol. The metachromatic cartilage shows up darkest in the photographs.

Fig. 1. Produced by a section of a tibia removed from a rat the thyroid of which had been destroyed by 100 μc. of iodine-131, administered on the 1st day of life.

Fig. 2. The tibia which produced this autoradiogram was removed from a rat which was similarly thyroidectomized by the use of iodine-131, subsequently given 5 γ of L-thyroxine daily for 14 days.

Fig. 3. Autoradiogram of a section of tibia from a control rat fed a low-iodine diet, the same diet on which the thyroidectomized rats were maintained after weaning.

Fig. 4. Autoradiogram produced by a section of tibia from a normal rat fed the stock diet.

Figs. 5, 6, 7, and 8. Photographs of stained sections which produced autoradiograms for Figs. 1, 2, 3, and 4.

Figs. 9, 10, 11, and 12 are autoradiograms of sections of tail skin from rats whose tibiae produced the autoradiograms shown as Figs. 1, 2, 3, and 4, respectively.
(Dziewiatkowski: Sulfomucopolysaccharides in thyroidectomized rats)
PLATE 8

Effect of surgical thyroidectomy on the uptake of $^{35}$-sulfate by tibiae. Autoradiograms produced by sections of the proximal ends of tibiae from 42-day-old rats. The tissues were fixed in a 10 per cent solution of formalin for 48 hours at 25°C. Kodak contrast process ortho film was exposed to the sections for 4 weeks. Each animal received per gm. of body weight 1 $\mu$g. of sulfur-35 as sodium sulfate 12 hours before sacrifice. $\times$ 5.

Directly to the right of an autoradiogram is the photograph of the section which produced it. The sections were stained with 0.1 per cent toluidine blue in 30 per cent ethanol before photography.

Fig. 13. Autoradiogram of a section of tibia removed from a rat the thyroid of which was surgically removed on the 28th day of life.

Fig. 14. The tibia which produced this autoradiogram was from a rat the thyroid of which was also surgically removed, but the rat subsequently received 5 $\gamma$ of L-thyroxine daily.

Fig. 15. Same as for Fig. 14, except that the rat received 10 $\gamma$ of L-thyroxine daily.

Fig. 16. Autoradiogram produced by a tibia from a rat subjected to a mock thyroidectomy and then fed the same low-iodine diet which was fed to the thyroidectomized rats.

Fig. 17. Produced by a section of tibia from a normal rat fed the stock diet.

Figs. 18, 19, 20, 21, and 22. Photographs of stained sections which produced autoradiograms for Figs. 13, 14, 15, 16, and 17.