The turnover of the acid mucopolysaccharides, hyaluronic acid (HA) and chondroitinsulfuric acid (CSA), of skin has been studied in normal rats (1) and in animals treated with the adrenocortical hormones, 17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone (2). It was shown that the administration of these adrenal steroids to otherwise normal rats resulted in a decreased turnover of the mucopolysaccharides of skin.

The possibility that insulin participates in the metabolism of acid mucopolysaccharides was suggested by the finding that both the uronic acid and hexosamine moieties of HA derive from glucose (3–5). Since insulin regulates the utilization of glucose, it seemed reasonable to postulate a role for insulin in the biosynthesis of the mucopolysaccharides. It is the purpose of this paper to present evidence that mucopolysaccharide metabolism is impaired in the skin of alloxan-diabetic rats and is restored toward normal by insulin administration. A portion of this investigation was published previously in preliminary form (6).

Methods and Materials

Animals—Adult male rats of the Sprague-Dawley strain were used throughout this study. Except for animals on a limited food intake, the rats were fed Rockland chow and water ad libitum. Alloxan diabetes was induced by a single subcutaneous injection of 150 mg. of alloxan monohydrate per kilo of body weight. Animals that did not lose weight were eliminated. 3 weeks after the administration of alloxan, blood glucose, determined on rats selected at random, ranged from 410 to 592 mg. per 100 ml.

Insulin (NPH, Lilly) was injected subcutaneously in daily doses of 20 or 40 units per kilo of body weight. This dose was varied, because continued administration of the higher dose resulted in evidences of hypoglycemia in some animals. Glucose levels of 25 to 45 mg. per 100 ml. of blood were

* This investigation was aided by grants from the National Heart Institute (H-311) of the United States Public Health Service, the Herman Gordon Foundation, and the Variety Club of Illinois.
Methods—Blood glucose was determined by the method of Nelson (7). C\textsuperscript{14}-carboxyl-labeled sodium acetate was prepared as described by Calvin et al. (8). Uniformly labeled glucose-C\textsuperscript{14} was purchased from the Nuclear Instrument and Chemical Corporation, Chicago. The HA and CSA fractions were isolated from the skin as described in an earlier publication (9).

Aliquots of the separated mucopolysaccharides were oxidized to CO\textsubscript{2} and the C\textsuperscript{14} was counted as BaCO\textsubscript{2} in an internal gas flow counter, corrected to infinite thickness. A silver wire used in the combustion tube filling assured the complete removal of the S\textsuperscript{35} during the oxidation of the CSA fractions. The S\textsuperscript{35} concentration of the CSA fractions was determined as previously described (1).

The HA and CSA pool sizes in the skin of normal and diabetic rats were estimated by the method of isotope dilution. C\textsuperscript{14}-labeled HA and CSA were prepared in vivo by injecting rats with acetate-1-C\textsuperscript{14} and were isolated from the skin as described (9). The specific activity of the two mucopolysaccharides was determined and known quantities of each were added at the beginning of the extraction procedure to NaOH suspensions of defatted ground skins from normal and diabetic animals. After isolation of the mixture of labeled and unlabeled mucopolysaccharides, the specific activities of the HA and CSA fractions were determined and the amounts of mucopolysaccharides in the original skins were calculated (10). The values for the pool sizes of HA and CSA are expressed as mg. per 100 gm. of acetone-defatted dry skin.

EXPERIMENTAL

60 rats were divided into three experimental groups of equal size. One group of animals was used 3 weeks after the administration of alloxan. A second group of untreated animals served as controls, while a third group of normal animals was maintained on half the average daily food intake for 3 weeks prior to and during the experiment. The weight loss of the latter group was similar to that of the diabetic animals throughout the experimental period. Each of the 60 rats was injected once subcutaneously with 80 \textmu c. of acetate-1-C\textsuperscript{14} and 2.7 \textmu c. of Na\textsubscript{2}S\textsuperscript{35}O\textsubscript{4} as an isotonic mixture. Ten rats in each group were sacrificed 1 and 5 days after the injection.

The uptake of C\textsuperscript{14} by HA and of C\textsuperscript{14} and S\textsuperscript{35} by CSA was strikingly less for diabetic than that for normal rats (Fig. 1). No such decrease was evident in the fasted rats. Since the weight lost by the animals on a restricted food intake duplicated that lost by the diabetic animals, weight...
loss per se appears to have no influence on the uptake of C\textsuperscript{14} and S\textsuperscript{35} by the mucopolysaccharides of skin. The average body weights of the three groups of animals at the time of isotope administration were 355, 212, and 210 gm. for the normal, fasted, and diabetic rats, respectively.

The disappearance of HA-C\textsuperscript{14}, CSA-C\textsuperscript{14}, and CSA-S\textsuperscript{35} 4 days after maximal labeling is illustrated in Fig. 1. The C\textsuperscript{14} concentration of the CSA fraction from the diabetic animals has been omitted, since the radioactivity of the BaCO\textsubscript{3} from this sample was too low for accurate counting. While half life times calculated from two points are not accurate, the values serve to indicate gross changes in turnover. The apparent half life times of 2.6 days for HA and 11.0 and 10.8 days for CSA (based on the C\textsuperscript{14} and S\textsuperscript{35}, respectively) found in the skin of normal animals agree with those obtained previously from more detailed decay curves (1, 2). In the diabetic animal, however, the turnover is considerably slower, as evidenced by an apparent half life of 4.5 days for HA and 20.9 days for CSA (based on S\textsuperscript{35}).

The results of this experiment indicated a decreased capacity to metabolize acid mucopolysaccharides in diabetic animals. A fall in concentration of these substances might therefore be anticipated. Since methods for isolating the mucopolysaccharides are not quantitative, attempts were
made to estimate possible changes in mucopolysaccharide concentrations by utilizing the method of isotope dilution. The results, indicated in Table I, demonstrate a marked decrease in HA concentration and a less striking decrease in CSA concentration. These determinations of pool size permitted the calculation of the turnover rates presented in Table I. The marked difference between diabetic and normal animals is evident. The lower turnover rates previously reported (6) were calculated from the quantities of HA and CSA isolated from skin by methods which are not quantitative.

The sizes of the HA and CSA pools in the skin of the fasted animals were not measured; hence turnover rates comparable to those calculated for the normal and diabetic rats could not be obtained.

**Table I**

*Comparison of Pool Size and Turnover Rate in Normal and Diabetic Rats*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pool size*</th>
<th>Turnover rate†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per 100 gm.</td>
<td>mg. per 100 gm. per day</td>
</tr>
<tr>
<td>HA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>215</td>
<td>58</td>
</tr>
<tr>
<td>Diabetic</td>
<td>88</td>
<td>14</td>
</tr>
<tr>
<td>CSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>187</td>
<td>12</td>
</tr>
<tr>
<td>Diabetic</td>
<td>145</td>
<td>5</td>
</tr>
</tbody>
</table>

* Pool size as determined by the isotope dilution method and expressed as mg. per 100 gm. of acetone-defatted dry skin.
† Turnover rate = (pool size)/(t₀ × 1.44) (11) (t₀ = half life time).

The use of acetate-1-C¹⁴ effects specific labeling of the N-acetyl component of the mucopolysaccharide molecules (12). Since acetate utilization is decreased in the diabetic animal (13, 14), it may be argued that the observed results reflect alterations in acetate metabolism. Although the similarity of the C¹⁴ and S³⁵ data would appear to invalidate this objection, another experiment was undertaken in which glucose-U-C¹⁴ as well as Na₂S³⁵O₄ was utilized. In the same experiment the effects of insulin on both normal and diabetic rats were studied. Four experimental groups were used. At appropriate times, as designated below, each animal received a single subcutaneous injection of an isotonic mixture containing 6.7 μe. of glucose-U-C¹⁴ and 13.3 μe. of Na₂S³⁵O₄. Two groups were made diabetic as described above. 3 weeks later one-half of the diabetic animals was injected with the radioactive mixture. The remainder was treated daily with 20 or 40 units of insulin per kilo of body weight for 1 week before the administration of the radioactive material and daily thereafter until sacrifice. The two other groups of animals consisted of non-
diabetic rats. One group served as a normal control, while the other was injected daily with 20 or 40 units of insulin per kilo of body weight before and after receiving the isotopes in a manner identical to that used in the treatment of the diabetic animal.

Eight to ten rats in each group were sacrificed at intervals of 1, 5, and 17 days after the administration of radioactive material and the HA and CSA fractions were isolated from the respective pools of skin.

A comparison of the left bars of the diabetic and normal groups (Fig. 2) indicates a marked decrease in incorporation of isotope in both HA and CSA in the diabetic animal. This finding is entirely in accord with the re-

![Fig. 2. A comparison of the C14 concentration of HA and of CSA of the various groups 1 (left bar) and 5 (right bar) days after the administration of glucose-U-C14 and Na2S35O4.](http://www.jbc.org/)

sults obtained when acetate-1-C14 was employed as a precursor. Calculation of half life times for HA again showed some prolongation in diabetic animals (5.0 days compared with 3.8 days for normal), although these differences were not as striking as those observed for CSA or those obtained in the previous experiment.

The sulfate data (Fig. 3) demonstrate, as in the previous experiment, a marked inhibition of isotope uptake in diabetic animals, although the differences in decay (half life times) are not as evident.

The administration of insulin to diabetic animals restores the defect of uptake toward normal as illustrated by the data for C14 in HA (Fig. 2) and for both C14 and S35 in CSA (Figs. 2 and 3). The half life times were actually shorter than normal in this group (HA-C14, 1.9 days; CSA-C14, 3.9 days; and CSA-S35, 6.3 days). This is not surprising, since these ani-
mals were hypoglycemic. The effect of insulin in normal animals was somewhat variable and not as striking as in the diabetic animals, probably because the diabetic animal is more sensitive to insulin.

**DISCUSSION**

In the present study an attempt was made to relate the pancreas to the synthesis of acid mucopolysaccharides. The data show that in diabetic rats the uptake of C\textsuperscript{14} by HA and of C\textsuperscript{14} and S\textsuperscript{35} by CSA is diminished, the sizes of the mucopolysaccharide pools in the skin are decreased, and the HA and CSA turnover rates are slower than normal. In the insulin-treated diabetic animal, the metabolism of the skin mucopolysaccharides assumes a more normal character.

The radioactivity at zero time of the HA and CSA fractions isolated from the skin of the diabetic animals was approximately one-third that of the same fractions isolated from the skin of the normal animals when either acetate-1-C\textsuperscript{14} or glucose-U-C\textsuperscript{14} and Na\textsubscript{2}S\textsuperscript{35}O\textsubscript{4} were administered. These substances are precursors of specific moieties of the HA and CSA molecules (1, 12). This fact, taken together with the finding that the uptake of the isotopes was reduced to the same extent with each precursor used, confirms and supports the conclusion published previously (1) that the various components of the respective acid mucopolysaccharides turn over at the same rate. Furthermore, the observations argue against the
view that an increased initial dilution due to larger than normal body pools of acetate and glucose is responsible for the results in the diabetic animal, for it is unlikely that the acetate, glucose, and sulfate pools were increased to exactly the same extent.

The results of this investigation indicate that the synthesis of the connective tissue mucopolysaccharides is inhibited in the insulin-deficient animal. That the decreased turnover of these substances in the diabetic rat results from a defect in glucose utilization seems likely. Such a concept would also account for the decreased turnover of the mucopolysaccharides in the skin of rats injected with adrenal cortical hormones (2).

Irrespective of its mechanism of action, a role of insulin in the metabolism of the acid mucopolysaccharides may have considerable biological implications. The retarded wound healing, the increased susceptibility to infection, and the accelerated vascular degeneration characteristic of diabetes mellitus may reflect a decreased ability to synthesize acid mucopolysaccharides.

**SUMMARY**

1. The turnover of the mucopolysaccharides, hyaluronic acid (HA) and chondroitin sulfate (CSA), was estimated in the skin of diabetic, fasted, insulin-treated non-diabetic and insulin-treated diabetic rats, following the administration of the precursors, acetate-1-C14 or glucose-U-C14 and Na2S35O4.

2. The uptake of C14 by HA and CSA and of S35 by CSA isolated from the skin of diabetic animals is approximately one third that found in the normal animals. Insulin treatment restored the values toward normal.

3. It is suggested that insulin participates in the metabolism of acid mucopolysaccharides.

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