The biosynthesis of mucopolysaccharides in the skin of alloxan-diabetic rats*

Substantial evidence has been advanced that the disturbance in the regulation of carbohydrate metabolism and the characteristically low store of liver glycogen in the diabetic subject can be restored with insulin treatment. Since it has been demonstrated that glucose is utilized for the synthesis of serum glucosamine by the rat[1] and for the synthesis of hyaluronic acid (HA) by the Group A streptococcus[2,3], it appeared plausible that insulin regulates the utilization of glucose for the synthesis of mucopolysaccharides in the ground substance of connective tissue. An investigation was undertaken, therefore, to study the effect of insulin on connective tissue mucopolysaccharides utilizing ^{14}C-labeled compounds and $Na_2{}^{35}SO_4$. The present communication is concerned with preliminary findings on the HA and chondroitinsulfuric acid (CSA) in the skin of diabetic rats and provides data indicating that insulin plays a role in the synthesis of these substances.

Adult male rats of the Sprague-Dawley strain were used throughout the experiment. Except for the animals on a limited food intake, the rats were fed Rockland chow and water *ad libitum*. The animals were divided into 3 experimental groups. Alloxan diabetes was induced in rats by a single subcutaneous injection of 150 mg of alloxan monohydrate per kg of body weight, as a 5% solution. A group of 20 rats was used 3 weeks after the diabetes was established. A second group of 20 untreated rats served as controls while a third group of 20 normal animals was maintained on half the average daily food intake for 3 weeks prior to and during the experiment. The weight loss in the latter group was similar to that in the diabetic animals over the 3 week period.

Each animal was injected once subcutaneously with 1.2 ml of a solution containing 80 μC of ^{14}C-carboxyl-labeled sodium acetate and 2.7 μC of $Na_2{}^{35}SO_4$, as an isotonic mixture. Ten rats in each group were sacrificed at intervals of 1 and 5 days after the injection. The animals were skinned and the HA and CSA fractions were isolated as described[4] from a pool of skins from 10 rats. The ^{14}C of the separated fractions was determined after oxidation to CO_2 in a microcombustion apparatus. A silver wire was used in the combustion tube filling to assure complete removal of ^{35}S.

The pertinent data are summarized in Table I. The amount of HA and CSA which could be isolated from the skin of diabetic rats was less than that from the non-diabetic groups, the decrease being more marked in the HA than in the CSA fraction. That HA appears to be more sensitive than CSA to an insulin deficiency is in keeping with the longer apparent half-life time[5] and the slower turnover rate of the latter compound in the skin of normal animals (Table I).

The incorporation of acetate by the HA and the CSA[5] in the skin of the diabetic rat was found to be about 1/3 that in the skin of either the normal or partially fasted animal. The ^{14}C in these fractions at zero time (24 h after injection) is shown in the accompanying table. A similar decrease was found in the uptake of ^{35}S by the CSA in the skin of the diabetic rats.

While the turnover rate of the skin HA is lower in the group of diabetic rats than in the other two groups, the turnover rate of the CSA appears to be unaffected.

The data presented suggest that the synthesis of the connective tissue mucopolysaccharides is inhibited in the insulin deficient animal. This finding may be an important factor in elucidating

TABLE I

THE MUCOPOLYSACCHARIDES IN THE SKIN OF NORMAL, FASTED AND DIABETIC RATS

Type of animal	*Aver. body wt.*	*Hyaluronic acid*			*Chondroitinsulfuric acid*		
(20 rats/group)	*g*	*Concentration mg/100 g dry skin*	*Radioactivity* C.P.M. at zero time*	*Turnover rate** mg day/100 g dry skin*	*Concentration mg/100g dry skin*	*Radioactivity* C.P.M. at zero time*	*Turnover rate** mg day/100 g dry skin*
Normal	355	59.6	6009	7.7	38.1	2032	2.4
Fasted	212	51.0	5811	9.3	55.3	1753	3.7
Diabetic	210	30.2	1968	4.6	25.6	769	2.3

* Refers to the ^{14}C in the group of 10 rats sacrificed 24 h after injection of radioactive acetate. All counts have been corrected for differences in body weight and are calculated on a per kg basis. All samples were counted as $BaCO_3$ in an internal gas-flow counter and were corrected to "infinite thickness".

** The turnover rate was calculated from the turnover time[6] as the average quantity of each mucopolysaccharide synthesized and degraded/day/100 g dry skin; where t_R = [polysaccharide]/t_t and $t_t = 1.44\, t_{\frac{1}{2}}$.

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the delayed wound healing, decreased resistance to infection and accelerated vascular degeneration that occurs in diabetes mellitus.

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The intracellular distribution of enzymes in *Serratia marcescens*

The localisation of enzymes on particulate components of cytoplasm in the cells of higher animals and plants is well established. It has been shown in *Serratia marcescens* that the red water-insoluble pigment, prodigiosin, could be isolated as a protein complex[1]. This would imply some intracellular organization. It became of interest therefore to see if it could be shown that in bacterial cells there occurs also an organised distribution of enzymes on different cell fractions. This preliminary note describes the distribution of a number of enzymes between particulate and soluble fractions obtained from this organism.

The organisms were disintegrated by a high-speed mechanical shaker (5600 cycles/min)[2]. The capactity of the capsule in which the cells were disrupted is 20 ml; most effective breakage was obtained with 15 g of Ballotini glass beads size No. 14 and 1.5 g wet weight of *S. marcescens* suspended in 10 ml of 0.8 *M* sucrose, leaving an air space of about 3 ml. The capsule and contents were cooled to 0° prior to shaking in the cell disintegrator for 15–20 seconds, the temperature during the shaking rising about 5°. Approximately 10% of the cells were disrupted. Oscillation for longer periods lead to greater cell breakage but a different pattern of enzyme distribution. After diluting with 1.5 volumes of 0.3 *M* sucrose and removing the glass beads by centrifugation the supernatant was centrifuged for 20 minutes at 3,500 *g* and the residue discarded, and this procedure repeated twice so that the supernatant was cell-free. All centrifugations were carried out at 0°. The supernatant was then made 0.17 *M* with respect to KCl and centrifuged for 20 minutes at 25,000 *g*; the sediment and supernatant were collected separately, and subsequent reference to the "supernatant" refers to this fraction. The sediment was then washed once with the original volume of 0.5 *M* sucrose containing 0.17 *M* KCl and the washed sediment finally suspended in 0.5 *M* sucrose for enzymic assay. Viewed in a phase-contrast microscope the sediment was heterogeneous, being composed of particles and debris. The sediment is hereafter referred to as the particulate fraction. The particulate fraction was not composed of cell nuclei as shown by analysis of the desoxyribonucleic acid (DNA) distribution; the DNA was exclusively in the supernatant. Whether the particulate fraction represents discrete bodies within the cell, or mainly fragments of cytoplasmic membrane cannot be answered.

The distribution of a number of dehydrogenases between the supernatant and particulate fractions is shown in Table I. A disproportionate arrangement of the enzymes between the supernatant and particulate fractions is evident from Table I. It is possible to divide the enzymes associated with the particulate fraction into two groups: the first contains enzymes which are firmly attached to the particulate fraction, and the second enzymes easily dissociable from the particulate complex. The first group of enzymes is composed of succinic, α-ketoglutaric, lactic and formic dehydrogenases. The distribution of this group was not greatly affected by the disintegration period, being almost entirely concentrated in the particulate fraction under all conditions. Aconitase, fumarase, isocitric and malic dehydrogenases comprise the second group of enzymes, and with these the shaking period and the concentration of sucrose in which the cells were shaken was critical.

These enzymes were found on the particulate fraction only with a period of shaking less than 30 seconds and when a sucrose concentration of 0.8 *M* was maintained. If the sucrose concentration was 0.5 *M* or less, no aconitase or fumarase could be detected in the particulate fraction and diminishing amounts of malic and isocitric dehydrogenases were found. The shaking period was even more critical, prolonged shaking rendering all of the enzymes in this group soluble.

The supernatant fraction, although containing appreciable concentrations of aconitase, fumarase, isocitric and malic dehydrogenases could not oxidise aerobically any of these substrates. Just as in the case of animal cells the terminal oxidase system of *S. marcescens* is exclusively associated with the