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THE ACTIVITY OF GOLGI-MEMBRANE GALACTOSYLTRANSFERASE
IN THE LIVER OF STREPTOZOTOCIN-DIABETIC RATS*

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The Golgi-rich membrane fraction isolated from streptozotocin-diabetic rat liver had a lower protein content than the corresponding fraction from normal liver. Its UDPgalactose-N-acetylgalactosamine galactosyltransferase activity calculated per 1 g of liver or whole liver was decreased. The electron-microscopic examination of the negatively stained fraction revealed morphological changes.

The morphology of the Golgi complex in thin sections of diabetic liver was also changed.

In juvenile diabetic patients, lower ratios of neutral sugars (Sarnecka-Keller et al., 1972b) and sialic acid (Sarnecka-Keller et al., 1976) to proteins were found in the seromucoid fraction, and also lower than in normal serum was the ratio of neutral sugars to proteins and the content of galactose in the non-diffusible glycopeptide fraction (Sarnecka-Keller et al., 1972a; Turyna et al., 1976). These results as well as those reported by Berenson et al. (1972) suggest that disturbances in incorporation of glycosyl residues into glycoproteins and glycopeptides occur both in natural and experimental diabetes. We suggested (Sarnecka-Keller et al., 1972a) that the lower ratio of neutral sugars to protein in glycopeptide fractions may be due to diminished activities of glycosyl transferases in diabetic liver. As the glycosylations proceed chiefly in Golgi complex, we determined the activity of UDPgalactose-N-acetylgalactosamine galactosyltransferase in the Golgi-rich membrane fraction originating from the livers of normal and diabetic rats.

MATERIAL AND METHODS

Animal material. The experiments were carried out on Wistar female rats, aged about six months and weighing 170 - 220 g. Both the control and diabetic rats were

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fed *ad libitum* on commercial pelleted food and tap water. The animals fasted for about 20 h were anaesthetized with ether, exsanguinated, their livers were isolated, weighed and used for preparation of the Golgi-rich membrane fraction.

Diabetes was induced by the injection of streptozotocin (55 - 65 μg/g body weight) into the tail vein. Those animals were considered diabetic which on the tenth or eleventh day showed a 3 to 7-fold increase in free sugar in blood as compared with the normal values. This was accompanied by a decrease in body weight averaging about 25%, and by polyuria and polydypsia.

**Reagents.** Tris, sodium 2-glycerophosphate, o-nitrophenyl-β-D-galactopyranoside, sodium cacodylate, 2-mercaptoethanol and bovine serum albumin were obtained from Koch-Light Lab. (Colnbrook, Bucks, England); streptozotocin came from Calbiochem (San Diego, Calif., U.S.A.), UDP-galactose and Triton X-100 from Sigma Chem. Co. (St. Louis., Mo., U.S.A.) and UDP-[U-14C]galactose (spec. act. 210 mCi/mmole) was obtained from the Radiochemical Centre (Amersham, England). Dowex 2x8 (200 - 400 mesh) came from Fluka A.G. (Buchs S.G., Switzerland), 5'-AMP from Roth Laborchem. (Karlsruhe, G.F.R.), ATP from Reanal (Budapest, Hungary), and glucose-6-phosphate, sodium or barium salts from Serva Feinbiochemica (Heidelberg, G.F.R.). N-Acetylglucosamine was prepared in the Department of Organic Chemistry of the Medical School in Białystok (Poland). Sucrose came from Reachim (U.S.S.R.); the remaining reagents were analytical grade products from P.O.Ch. (Gliwice, Poland).

**Preparation of Golgi membranes.** Golgi-rich fractions were isolated from whole rat liver homogenates by the single-step sucrose-gradient procedure elaborated by Fleischer & Fleischer (1970). The fractions were collected from sucrose interphase between 1.14 - 1.12 g/cm³. Their purity was checked by electron microscopy as well as by estimation of RNA content and determination of activities of some marker enzymes.

**Electron microscopy.** The Golgi-rich membrane fractions were negatively stained, and liver thin sections were fixed according to Sturgess *et al.* (1973). The preparations were examined in a Philips EM-300 electron microscope.

**Assays of marker enzymes.** The suspensions of Golgi-rich fractions were three times frozen and defrozen before use. As a rule, the activities of the marker enzymes were determined 48 h after preparation of the Golgi membranes. 5'-Nucleotidase (EC 3.1.3.5) was assayed according to Song & Bodansky (1967), Mg²⁺-stimulated ATPase (EC 3.6.1.3) according to Fleischer & Fleischer (1967), acid phosphatase (glycerol-2-phosphatase, EC 3.1.3.19) according to Bodansky *et al.* (1933), and glucose-6-phosphatase (EC 3.1.3.9) according to Swanson (1955). β-Galactosidase (EC 3.2.1.23) was determined by the method of Hughes & Jeanloz (1964) with some modifications introduced by Fleischer & Fleischer (1969) for hepatocyte plasma membranes.

**Assay of galactosyltransferase.** The activity of this enzyme was determined by the method of Babad & Hassid (1966) as modified by Fleischer *et al.* (1969), immediately after isolation of the Golgi-rich membrane fraction by single-step sucrose-gradient centrifugation. UDP-[14C]galactose was used as the donor, and N-acetyl-
glucosamine as the acceptor of the galactose residue. The radioactivity was determined in Packard Tri-Carb liquid scintillation spectrometer (model 2111). Two controls were performed: one for enzymatic hydrolysis of UDP-galactose (without the acceptor), and the second for non-enzymatic hydrolysis (without the Golgi-rich membrane fraction), and the values obtained were subtracted.

Analytical methods. Blood sugar was estimated by the method of Somogyi & Nelson (1962). To determine the RNA content in the Golgi preparation, pentose was assayed by the orcinol method (Mejbaum, 1939) in the extract from trichloroacetic acid-insoluble material obtained according to Schneider (1957). Protein was determined by the method of Lowry et al. (1951) with crystalline bovine serum albumin as a standard, and inorganic phosphorus according to Fiske & Subbarow (1925).

RESULTS

Negative staining of the Golgi-rich membrane fractions showed high purity of these preparations. The electron micrographs (Fig. 1a,b) of the fractions from normal rat liver revealed well preserved cisternae, vesicles and the tubular network, and only traces of contamination by fragments of other membranes. In the Golgi preparations from diabetic liver, preserved cisternae were never found, and fragmentation of tubular network and altered vesicle forms were observed (Fig. 2a,b).

The electron micrographs of thin sections of liver showed that the normal organization of the Golgi complex observed in control rats (Fig. 3a) is also altered in diabetic tissue (Fig. 3b). Out of 100 Golgi complexes observed in situ, 90 had abnormal shapes in diabetic liver, whereas in normal liver such drastic deformations were never found. In diabetic liver, the Golgi cisternae were always very curved, often nearly circular, without peripheral bulbs, and the vesicles were translocated.

The amount of the Golgi-rich membrane fraction isolated from diabetic liver (expressed in milligrams of protein per 1 g of liver) was only about one-fourth that obtained from normal liver (Table 1).

Table 1

The amount of Golgi membranes in preparations isolated from livers of normal and streptozotocin-diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Diabetic group</th>
<th>t</th>
<th>P</th>
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<tbody>
<tr>
<td>n=9</td>
<td>n=8</td>
<td></td>
<td></td>
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<tr>
<td>Liver weight (g)</td>
<td>4.5 - 6.5</td>
<td>4.5 - 6.5</td>
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<tr>
<td>Homogenate (mg protein/g liver)</td>
<td>200.8 ±119.1</td>
<td>140.4 ±30.6</td>
<td>1.39</td>
<td></td>
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<tr>
<td>Golgi membranes (mg protein/g liver)</td>
<td>1.21 ±0.51</td>
<td>0.31 ±0.24</td>
<td>4.52</td>
<td>&lt;0.001</td>
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</table>
It should be noted that of the two adjacent fractions obtained by sucrose-density-gradient centrifugation from the diabetic liver homogenate, the fraction of higher density also showed a smaller content of protein as compared with normal liver; similarly, the fraction of lower density, composed chiefly of phospholipids, was diminished.

Table 2
Specific activity of marker enzymes and RNA/protein ratio of the Golgi-rich membrane fraction isolated from livers of control and diabetic rats

The activity of phosphatases is expressed as μmoles of released Pi/min/mg protein, and that of galactosidase as μmoles of o-nitrophenol released from o-nitrophenyl-β-D-galactopyranoside/h/mg protein. ATPase and galactosidase were assayed at 32°C, and the other enzymes at 37°C. In parentheses the limit values are given.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=4)</th>
<th>Diabetic group (n=3)</th>
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<tbody>
<tr>
<td>5'-Nucleotidase</td>
<td>0.089 (0.048 - 0.131)</td>
<td>0.043 (0.020 - 0.076)</td>
</tr>
<tr>
<td>Mg²⁺-stimulated ATPase</td>
<td>0.281 (0.091 - 0.924)</td>
<td>0.593 (0.053 - 1.400)</td>
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<tr>
<td>Acid phosphatase</td>
<td>0.030 (0.015 - 0.067)</td>
<td>0.008 (0.002 - 0.024)</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>0.025 (0.000 - 0.050)</td>
<td>0.013 (0.000 - 0.040)</td>
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<tr>
<td>Galactosidase</td>
<td>49.5 (47.0 - 52.0)</td>
<td>248.0 (102.0 - 380.0)</td>
</tr>
<tr>
<td>RNA/protein weight</td>
<td>0.156 (0.098 - 0.208)</td>
<td>0.367 (0.098 - 0.738)</td>
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</table>

The specific activities of some marker enzymes and RNA/protein ratio of the Golgi membrane preparation from normal rat liver (Table 2) are similar to the corresponding values reported by other authors (Fleischer et al., 1969; Morre et al., 1969, 1970; Merrit & Morre, 1973; Bergeron et al., 1973; Kataoka & Moscarello, 1975) in spite of the higher yield of the Golgi-membrane-rich fractions as compared

Table 3
Activity of UDPgalactose N-acetylglucosamine galactosyltransferase in the Golgi-rich membrane fraction isolated from the livers of control and streptozotocin-diabetic rats

The activity is expressed as μmoles of galactose transferred per hour. Mean values ± S.D. are given, with limit values in parentheses.

<table>
<thead>
<tr>
<th>Activity calculated per:</th>
<th>Control group (n=9)</th>
<th>Diabetic group (n=8)</th>
<th>t</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>1 mg of protein of Golgi fraction</td>
<td>76 ± 43.8 (24.4 - 169.5)</td>
<td>156 ± 79.4 (59.0 - 265.5)</td>
<td>4.46</td>
<td>&lt;0.001</td>
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<tr>
<td>1 g of liver</td>
<td>104 ± 46.0 (67.4 - 187)</td>
<td>29 ± 3.3 (11.8 - 44.5)</td>
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<tr>
<td>whole liver</td>
<td>601 ± 284 (350 - 1104)</td>
<td>174 ± 31.5 (81.0 - 311.5)</td>
<td>4.07</td>
<td>&lt;0.001</td>
</tr>
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</table>
Fig. 1. Electron micrographs of negatively stained preparations of Golgi-rich membrane fraction isolated from livers of two control rats:
a, preserved part of cisternae and vesicles; b, typical arrangement of the perforated part of the cisternae.

M. Śmierko-Keller & A. Kordowiak (facing p. 182).
Fig. 2. Electron micrographs of negatively stained preparations of Golgi-rich membrane fraction isolated from livers of two streptozotocin-diabetic rats (a and b). In both cases, disarrangement of the Golgi membranes and fragmentation of the structures typical of this organelle are visible.

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Fig. 3. Electron micrographs of thin section from a, normal rat liver and b, streptozotocin-diabetic rat liver.

In a, in the vicinity of bile cannula (BC) is visible a stack of parallel cisternae with peripheral bulbs, and large secretory vesicles (SV) are visible on the distal face of the Golgi complex. Arrows point to groups of small vesicles. In b, cisternae are circularly curved and without peripheral bulbs. Secretory vesicles are absent and small vesicles (arrows) are located unequally in the vicinity of cisternae.

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The altered morphology of the Golgi fraction is in agreement with the reported observations concerning cytoplasmic and plasma membranes in experimental diabetes. In alloxan-diabetic rat liver a dispersion of polyosomes was accompanied by a lower rate of protein biosynthesis (Pilkis & Korner, 1971), and a decreased amount of rough endoplasmic reticulum was also found (Morgan & Jersild, 1970; Stenram & Wielander, 1973). In alloxan-diabetic rats, in heart cells (Marinari et al., 1974) and in hepatocytes (Harano et al., 1972; Reaven et al., 1973; Schmidt et al., 1974), there was a lower number and structural changes of mitochondria, and the activities of the mitochondrial membrane-bound enzymes were altered. Stenram & Wielander (1973) and Chandramouli & Carter (1975) found changes in the activity of some enzymes in the liver cell plasma membrane of streptozotocin-diabetic rats; the lowered activity of 5'-nucleotidase corresponds with the diminished activity of this enzyme in the Golgi preparation (Table 2). However, with the exception of galactosyltransferase, the number of individual determinations of marker enzymes is in our experiments too small to take the results obtained as conclusive evidence.

It is of interest whether the biochemical and morphological changes observed in the Golgi preparations are due only to the diabetes induced by streptozotocin, which injures β-cells, or are also due to the direct effect of streptozotocin on the Golgi membranes. This seems possible as Sturgess et al. (1974) observed structural disarrangement of the Golgi complex on administration of aminonucleoside of puromycin.

The electron microscopic examinations were performed in the Electron Microscope Laboratory in the Pediatric Institute of the Medical School in Cracow. The authors wish to express their gratitude to Dr. F. Kaczmarski for his help in the interpretation of electron micrographs.

REFERENCES


**AKTYWNOŚĆ TRANSFERAZY GALAKTOZYLOWEJ WE FRAKCIJ WZBOGACONEJ W BŁONY GOLGIEGO, IZOLOWANEJ Z WĄTROB SZCZURÓW Z CUKRZYCAW Y WYWOŁANĄ STREPTOZOTOCYNĄ**

**Streszczenie**

Frakcja wzobogacona w błonę Golgiego, izolowana z wątroby szczurów z cukrzycą wywołaną streptozotocyną, zawiera mniejszą ilość białka niż analogiczna frakcja pochodząca z wątroby zwiezrzyżnych kontrolnych. Aktywność galaktozylotransferazy (z akceptorem N-acetyloglukozaminy) tej frakcji, wyrażona na 1 g wątroby lub na całą wątrołę, jest obniżona.

Badania w mikroskopii elektronowej barwionej negatywowo frakcji wykazały zmiany morfologiczne w odniesieniu do kontroli. Morfologia aparatu Golgiego w cienkich skrawkach pochodzących z wątroby cukrzycowej była również zmieniona.

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