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Sodium Metavanadate Affected Control and Streptozotocin-Diabetic Rat Liver Golgi Complexes

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As we have observed previously, rat liver Golgi complexes are very useful cell organelle in investigating the effectiveness of various drugs with cytoprotective or normalizing activities in streptozotocin (STZ)-induced diabetes. The diabetes-associated biochemical and morphological alterations in the organelle were investigated in four groups of rats: control and STZ-diabetic (C and D groups) and compared with the same groups treated with 1.5 mM metavanadate administered as drinking solutions in 0.09 M NaCl over 7 days (C+V and D+V groups). Apart from the untreated group C, a decrease of body weight during the experiment was noted in the remaining three groups, reaching a statistically significant level in the diabetic groups (c. 15%). Fluid and food intake were statistically significantly ($p<0.001$) limited in both the vanadium treated groups. In the diabetic group treated with metavanadate, the free blood sugar level decreased, but euglycemia was not achieved. In groups C+V, D and D+V, the activity of Golgi marker enzyme, i.e. galactosyltransferase (GalT), was statistically lower as compared with group C ($p<0.001$). The treatment of diabetic rats with 1.5 mM $NaVO_3$ [V(V)] in 0.09 M NaCl as a drinking solution during 7 days did not normalize the yield of Golgi membrane preparations or the Golgi marker enzyme activity. Electron microscopy revealed marked ultrastructural changes triggered by the employed vanadium compound. A striking change was seen in the presence of giant intracytoplasmic vacuoles. These alterations were seen in both experimental groups, i.e. C+V and D+V. Group C+V showed more advanced ultrastructural changes, what was expressed in a poorer state of mitochondrial membranes, a greater number of vesicular structures and less frequently seen Golgi structures. In spite of the fact that the animals were exposed to two compounds with a strong biological activity, the group of diabetic rats treated with metavanadate (D+V) showed no such advanced changes: more numerous Golgi structures were noted and their form was practically ring-like, i.e. characteristic for this group of organelles.

Introduction

Among dozens vanadium compounds studied as insulin-mimetic drugs [2, 5, 9, 16, 24, 26, 27, 33, 35, 39], the most frequently used inorganic derivatives are vanadium sulphate [V(IV)] [1, 11, 14] and meta- and orthovanadate [V(V)] [2, 6, 10, 26, 30], employed both in diabetic models and in human volunteers. The relationship between the structure and function of the physiology, biochemistry and morphology of organs, tissues and cells in control and diabetic animals exposed to vanadium effect, seems to be of special interest. This problem, contrary to physiology and biochemistry, is relatively poorly understood. Several years ago, we found that biochemical and morphological alterations of diabetic rat liver Golgi complexes [29] were so characteristic that we used them as a test in investigating the effectiveness of drugs with cytoprotective and diabetes-normalizing properties [7, 8, 17–21]. In the cell, the Golgi complex is the organelle responsible not only for modification of newly synthesized macromolecules, but also for segregation and participation in subcellular traffic and remodeling of intracellular membranes. The role of this organelle in biosynthesis of glycosyl parts both secretory and membrane glycoproteins are very important in diseases associated with the metabolism of sugars, especially in diabetes.

In our experimental models, of seven investigated vanadium compounds (four organic and three inorganic), the drug that showed the best control of diabetic symptoms in liver Golgi complexes was an organic derivative, bis(maltola-

to)oxovanadium(IV) [BMOV] [7,20]. The present report describes the effect of an inorganic vanadium salt [V(V)], i.e. 1.5 mM sodium metavanadate, in 0.09 M NaCl as a drinking solution during seven days.

Material and Methods

Animals

The experiments were carried out in four groups of female Wistar rats, approximately 6 months old and weighing 200–250 g, by a permission of the Cracow Ethics Commission for Animal Experiments. The animals were fed with standard peleted food and given tap water prior to the experiments. The rats were not starved prior to the experiments to reduce effects other than experimental conditions.

Group C – control rats that received 0.09 M NaCl as a drinking solution for 7 days (6 rats),

Group C+V – rats that received 1.5 mM solution $NaVO_3$ in 0.09 M NaCl for 7 days (8 rats),

Group D – diabetic animals, receiving 0.09 M NaCl as a drinking solution for 7 days (6 rats),

Group D+V – diabetic rats that received 1.5 mM $NaVO_3$ in 0.09 M NaCl as a drinking solution for 7 days (10 rats).

In the two latter groups, diabetes was induced by an intraperitoneal streptozotocin (STZ) injection, in a dose of 60 mg STZ per kg of body weight, freshly dissolved in 0.05 M citrate buffer (pH 4.5). Animals, which on the third day had free blood sugar level above 13.9 mmol/l (250 mg/100 ml) were used in further experiments as diabetic rats. The animals received 0.09 M NaCl or 1.5 mM $NaVO_3$ dissolved in 0.09 M NaCl (to diminish its toxicity) as described below; the solutions were freshly prepared every two days. All animals in four groups survived the experiments.

All the animals were weighed each day, and the amounts of drinking liquids and food consumed by the rats were measured. At the end of the study, liver samples for morphological analysis were taken under anesthesia, the livers were immediately used for the isolation of Golgi membranes and the estimation of the GalT activity according to Fleischer [12] was performed.

Analytical methods

Protein was estimated by the method of Lowry et al. [23] with crystalline serum bovine albumin as a standard. Free blood sugar level was estimated according to Somogyi and Nelson [31]. The Golgi-rich membrane fraction was isolated, and the galactosyltransferase (GalT) activity estimated according to the Fleischer [12] method.

Ultrastructural examination

For electron microscopy, two to four biopsy materials from each group were fixed overnight in formaldehyde-glutaraldehyde fixative at 4°C by the method of Karnovsky [15]. The tissue was subsequently postfixed in 1% osmium tetroxide. After dehydration in graded concentrations of ethyl alcohol and propylene oxide, the tissue was embedded in the Spurr medium. Samples were sectioned with an ultramicrotome Reichert Ultracut S using a diamond knife. Semi-thin sections were stained with methylene blue and ultra-thin sections with 8% uranyl acetate dissolved in 50% methanol and then in lead citrate according to Venable and Coggeshal [34]. All studies were performed under an electron microscope Zeiss EM 900 operating at 80 kV. Primary magnification of the electron microscopic (EM) examinations was 20,000×.

Statistical analysis

All the results expressed as mean ± SD were tested for statistical significance by the Student's t-test. Statistically significant ($p<0.05$) values are marked under the table or figure.

Reagents

Sodium cacodylate, serum bovine albumin, Folin phenol reagent, UDP-Gal, Triton X-100, streptozotocin, uranyl acetate, lead citrate, TRIS and β-mercaptoethanol came from Koch-Light Lab. Dowex 2×8 with granulation (200–400 mesh) came from Fluka&Buchs, ^{14}C UDP-Gal with the specific activity of 292 mCi/mmol was obtained from Radiochemical Centre Amersham. All other reagents were purchased as analytical grade from Polish Reagents POChem Gliwice. For electron microscopy, Spurr epoxy resin from Pellco Co and formaldehyde, glutaraldehyde and osmium tetroxide were purchased from Polysciences Inc.

Results

In the three experimental groups (C+V, D and D+V), a decrease of body weight during the experiment was noted. The drop in body weight was statistically significant as compared to the controls – in untreated diabetes ($p<0.01$) and in vanadium-treated diabetes ($p<0.01$). In both the vanadium treated groups (C+V and D+V), the liver weight was lower than in group C ($p<0.02$ and $p<0.01$, respectively). Fluid and food intake were limited in both the vanadium treated groups ($p<0.01$ or $p<0.001$). In the untreated diabetic rats (group D), this parameter increased in a statistically significant way as compared to the control group ($p<0.001$); the symptom is very well known and characteristic of diabetes. In group D, the free

TABLE 1

Physiological and biochemical characteristics of investigated animals. A detailed description of the investigated groups of rats is supplied in Material and Methods

		C n = 6	C + $NaVO_3$ (C+V) n = 8	D n = 6	D + $NaVO_3$ (D+V) n = 10
Body weight during experiment [g]	Start	222.5 ± 15.5	243.4 ± 21.2	220.1 ± 18.4	243.0 ± 20.6
	End	284.1 ± 58.8	239.2 ± 24.0	186.2 ± 14.0 a)	205.3 ± 21.9 b)
	% of change	19.4 ± 13.3 ↑	1.8 ± 2.1 ↓	15.3 ± 4.1 ↓	15.5 ± 5.6 ↓
Liver weight [g]		9.0 ± 1.1	7.6 ± 0.7 c)	8.1 ± 1.2	6.9 ± 1.1 e), d)
Fluid intake [ml/rat/day]		27.0 ± 6.6	15.6 ± 2.2 f)	124.9 ± 12.1 g)	20.6 ± 2.1 h)
Food intake [g/rat/day]		17.9 ± 0.9	15.3 ± 1.2 i)	28.1 ± 2.4 k)	10.3 ± 2.1 l)
Free blood sugar level [mg/100ml]	3-rd day after STZ	–	–	396.6 ± 119.9	326.9 ± 33.4
	Last day of experiment	126.5± 18.8	165.6 ± 39.0	416.7 ± 132.1 m)	304.9 ± 52.9 n)
Yield of Golgi-rich fraction [mg of protein/g of liver]		0.347 ± 0.122	0.325 ± 0.090	0.225 ± 0.137	0.226 ± 0.090 o)
GalT activity [nmoles Gal/h, g of liver]		93.5 ± 24.9	31.1 ± 14.5 p)	24.6 ± 13.5 r)	28.5 ± 22.3 s)
Total GalT activity [nmoles Gal/h, total liver]		848.9 ± 265.3	241.1 ± 131.3 t)	203.8 ± 103.9 u)	207.1 ± 186.1 w)

As compared with C group:
a) p<0.01; b) p<0.01; c) p<0.02; e) p<0.01; f) p<0.001; g) p<0.001; h) p<0.02, i) p<0.01; k) p<0.001; l) p<0.001; m) p<0.001; n) p<0.001; o) p<0.05; p) p<0.001; r) p<0.001; s) p<0.001; t) p<0.001; u) p<0.001; w) p<0.001
As compared with D group:
d) p<0.02; h) p<0.001; l) p<0.001; n) p<0.05

blood glucose level increased during the experiment. In the vanadium treated diabetic group (D+V), it was decreased, but euglycemia was not achieved (Table 1).

The yield of isolated Golgi-enriched membrane fraction was lower in groups D and D+V in comparison with group C (the last p<0.05). In the three experimental groups: C+D, D and D+V, the activity of Golgi marker enzyme i.e. galactosyltransferase (GalT), was statistically significantly lower than in the controls, regardless of the mode of expression (p<0.001).They were lower when calculated as nmoles Gal transferred per 1 h and per 1 g of liver or as nmoles Gal transferred per 1 h and per whole liver, as total glycosylation possibility in this organ (Table 1). A similar dispersion of individual results in the three experimental groups of rats is shown in Figure 1.

Electron microscopy revealed the presence of marked ultrastructural changes, most likely evoked by the effect of the employed vanadium compound. A striking alteration was the presence of giant, intracytoplasmic vacuoles. Such changes were noted in both groups exposed to vanadium, i.e. in C+V and D+V groups. Group C+V demonstrated somewhat more advanced ultrastructural changes, visible in mitochondrial membranes that were more poorly preserved, an increased number of vesicular structures and less frequent Golgi structures. The latter were seen as 2–3 narrow,

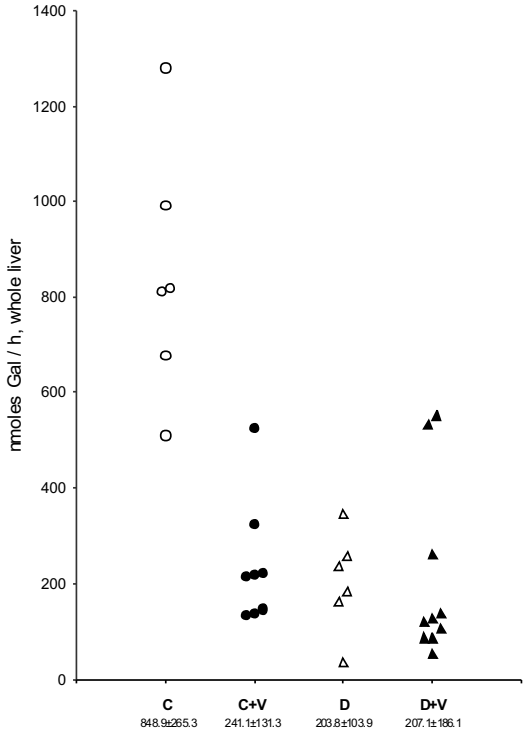
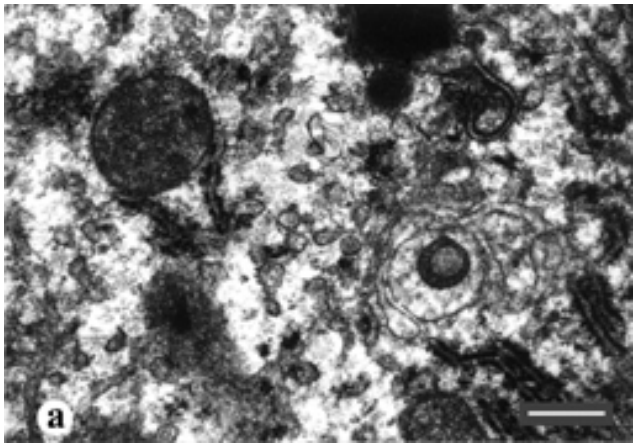
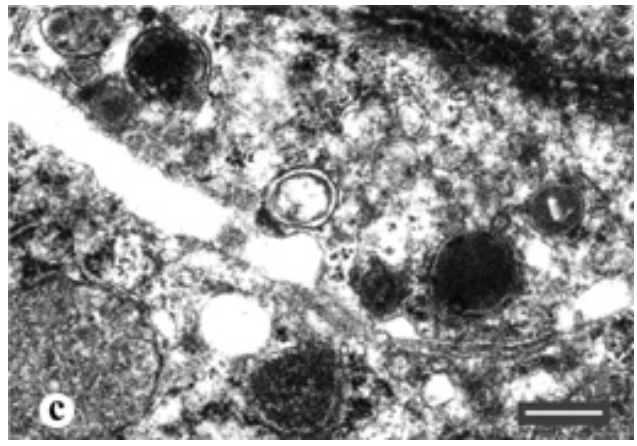


Fig. 1. Dispersion of results of estimated total galactosyltransferase activity (expressed as nmoles Gal transferred per 1 hour and per whole liver). The mean values ±SD in all study groups are given. In all experimental groups, a statistically significant lower activity of the enzyme in comparison with the control was noted (p<0.001). p values are given also below





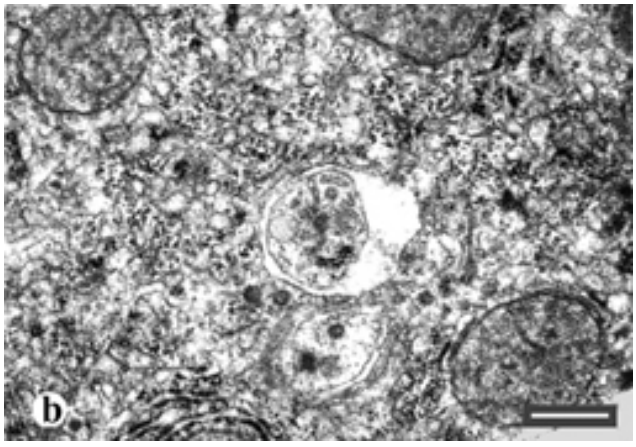


Fig 2. C+V group. The bar represents 0.4 μm: Fig. 2a. A Golgi complex in the form of two arched cisterns and 2–3 vacuoles with a common axis. Note numerous vesicles in the vicinity. Fig. 2b. Note a Golgi complex with a single, grossly distended cistern situated in the "cytoplasmic bridge" between vacuoles. Numerous coated vesicles. Mitochondria show slightly changed external membranes. Fig. 2c. Two adjacent Golgi complex form a sort of a continuum. Straight, narrow cisterns with marginal distensions, one of them of unparalleled length. Lysosome-like structures are seen in the vicinity.

slightly arched cisterns, which were at times twisted, forming semi-annular shapes (Fig. 2a). In their vicinity, coated and small electron-lucid vesicles were observed. At times, giant vacuoles were noted in the immediate neighborhood, being either empty or showing cytoplasmic structures encapsulated by membranes (Fig. 2b). This group manifested Golgi complexes having a form of two narrow cisterns with distended marginal terminals, one of which ended with a very long, distended terminal (Fig. 2c).

In diabetic animals exposed to vanadium (group D+V), the ultrastructure showed no such advanced changes. Numerous Golgi structures were seen, their form being practically annular and characteristic for this group. In this group, Golgi structures were usually accompanied by numerous electron-dense, membrane-covered lysosome-like structures (Fig. 3a). In the location typical for Golgi structures, i.e. in the vicinity of biliary canaliculi, the authors observed multiple forms of annular Golgi structures. At times, two ring-like structures were encircled by a single, common cistern. Also in this case, in their vicinity numerous electron-lucid vesicles were noted, while coated vesicles were less frequent and lysosome-like structures were abundant (Fig. 3b). Cytoplasmic regions forming "bridges" protruding between giant vacuoles filled with electron-lucid, floccular material showed annular Golgi complexes in their typical location. The central cistern contained electron-dense material. In the vicinity, lysosome-like structures were observed (Fig. 3c).

Discussion

Metavanadate [V(V)] as 1.5 mM solution administered over during 7 days to STZ-diabetic rats did not normalize blood glucose levels, as it caused only a 7% decrease. Under the same experimental conditions, Na_3VO_4 caused a 23% drop, whereas 3 mM orthovanadate decreased this parameter by 60%. 3 mM vanadyl sulphate, in which vanadium appeared as [V(IV)], caused the reduction of free blood sugar level by approximately 38% [8, 21]. Na_3VO_4 in 3 mM concentration, however, showed a very good effect in normalizing glucose blood sugar levels, but resulted in high mortality (67%) in the investigated rats [23]. Two other vanadium inorganic compounds, as well as 1.5 mM orthovanadate, did not cause any mortality in experimental rats [8]. Body and liver weight were decreased in comparison with the untreated C group in all the investigated groups of rats. The administration of sodium metavanadate as a 1.5 mM drinking solution caused a reduction in fluid and food intake, simi-

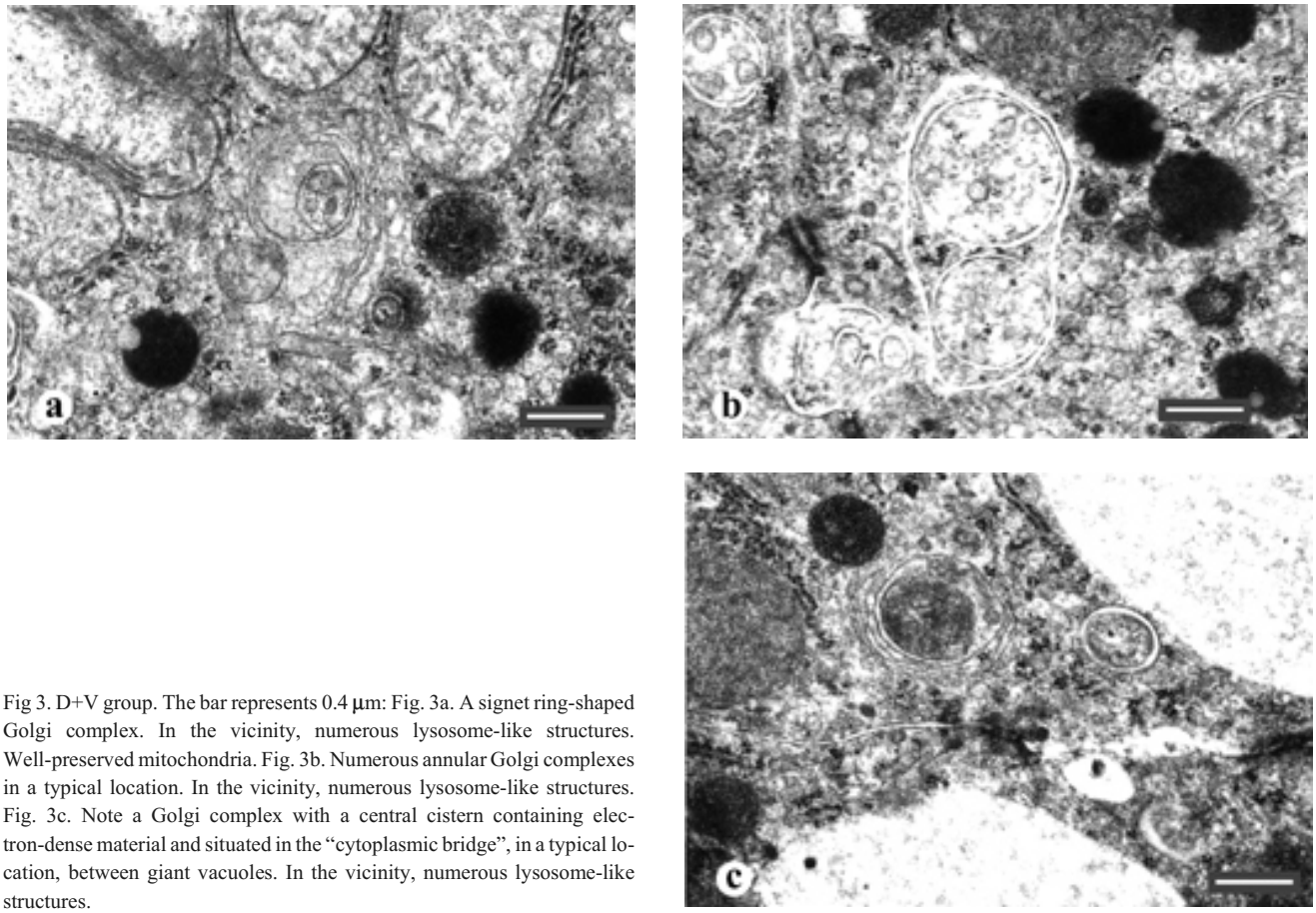


Fig 3. D+V group. The bar represents 0.4 μm: Fig. 3a. A signet ring-shaped Golgi complex. In the vicinity, numerous lysosome-like structures. Well-preserved mitochondria. Fig. 3b. Numerous annular Golgi complexes in a typical location. In the vicinity, numerous lysosome-like structures. Fig. 3c. Note a Golgi complex with a central cistern containing electron-dense material and situated in the "cytoplasmic bridge", in a typical location, between giant vacuoles. In the vicinity, numerous lysosome-like structures.

larly as in the case of all vanadium derivatives. This observation is in agreement with our previous study [8, 17–21] and with results achieved by other authors [3, 4, 22, 25, 28, 32, 36–38], and the reduction was especially pronounced in the vanadium-treated diabetic group D+V as compared to the untreated diabetic D rats. In the case of other investigated vanadium fluids, the intake was reduced 5-8 times, while in the case of metavanadate, the reduction was approximately sixfold.

The yield of liver Golgi-rich membrane fraction was the same in controls (vanadium treated and untreated) groups. In two diabetic groups this parameter was statistically significantly lower than control. The activity of galactosyltransferase, the Golgi marker enzyme, which expressed glycosylation ability of the liver, was statistically significantly ($p<0.001$) lower as compared with control value in the untreated group D; however, after the treatment with other vanadium compounds was intensified, the control level (excluding the action of BMOV) was not achieved. A similar dispersion of individual results after metavanadate treatment or untreated diabetes in the three experimental groups (C+V, D and D+V) was noted. Metavanadate treatment in concentration of 1.5 mM did not normalize the GalT activity. The same effects were observed in the case of vanadyl sulphate and two concentrations of orthovanadate [8,21]. The total galactosyltransferase activity in group D+V increased as compared with group D also after the administration of four organic vanadium complexes [7, 17–19], but only BMOV normalized this value [20].

The Golgi complex plays an important role in segregation inside the cell, biosynthesis and modification of biomolecules, especially in glycosylation process, and in biogenesis of some cell organelles, as well as maturation and alterations of intracellular membranes. According to this different role, structure of the Golgi complex is composed [13]. Additionally, this organelle is sensitive to the effect of various drugs and its morphology is associated with changes of its biochemical activity. In our investigations, we have encountered such markedly advanced structural changes for the first time. These alterations do not display dystrophic character. Giant vacuoles assuredly originate from membranous structures, yet we are unable to determine from which compartment they are derived and whether the Golgi structures are directly involved in their formation. The Golgi complex, as a "membrane manufacturer", obviously may be involved in an indirect way. As it is well-known, marginal distensions detach themselves from the Golgi complexes as large, electron-lucid vacuoles. In our material, we have observed cisterns with marginal distensions of monstrously elongated shape. Such structures,

when combined with similar ones, may form such giant vacuoles. A great number of vesicles that fill the cytoplasmic regions may originate from the transformation of the smooth reticulum canaliculi. Yet the vesicles situated in the vicinity of the Golgi complexes in less altered regions of the cells belong to the organelle. The shape of the Golgi complex itself obviously depends on the angle of section, but oval structures, originating from a tangential (horizontal) section of a normal Golgi complex can be easily differentiated from spatially altered Golgi structures. These cylindrical forms of Golgi complexes markedly predominate in groups of diabetic animals. Often they appear as numerous, scattered complex structures. Many times we have noted in this group – in spite of (or possibly because of) the fact that the animals were administered two different substances, i.e. STZ and various vanadium derivatives – that ultrastructural changes of the hepatocytes are less diversified or less intensified.

It appears that the normalizing effect of metavanadate (as well as other investigated vanadium derivatives) is manifested in „diabetic" cells" of the hepatocytes, while in normal cells, the effect mostly triggers destructive changes.

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