Pol J Pathol 2004, 55, 1, 25-32 PL ISSN 1233-9687

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Influence of Vanadyl Sulphate [$VOSO_4$] on Biochemical Activity and Morphology of Control and Streptozotocin-Diabetic Rat Liver Golgi Complexes

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The authors describe the influence of vanadyl sulphate on liver Golgi complexes in control and streptozotocin (STZ)-diabetic rats. $VOSO_4$, one of inorganic vanadium compounds widely used in animal models and human diabetes, acts as an insulin-mimetic drug and is relatively well known as a complex activated or inhibited on many enzymes involved in carbohydrate or lipid metabolic pathways. A relatively small in scope investigation was performed on subcellular levels, while changes of Golgi complexes under vanadium influence have not been described with the exception of our previous investigations with four organic derivatives. This paper presents the action of vanadyl sulphate used in 3mM in 0.5% NaCl as a drinking solution for 7 days on control and STZ-diabetic rat liver Golgi complexes. Changes induced by this vanadium compound were greater in the controls as compared to the diabetic rats, what was true for both biochemical and morphological data. Physiological and biochemical analyses showed a partial normalization of the investigated parameters in diabetic animals after short time treatment with vanadyl ions, although STZ-diabetic, vanadium treated rats were affected by two types of adverse effects exterted by these compounds. The controls manifested more numerous and advanced subcellular changes. The moderately developed Golgi apparatus showed no major changes. In the control group, subcellular changes were seen sporadically. More extended Golgi complexes showed certain anomalies.

Introduction

Diabetes, a social disease with a tendency to increase (with the incidence of 140 millions people worldwide in 2000) and a high death index (in North America approximately 400000 per year) [35] needs special efforts to find new, anti-diabetic drugs . These drugs should be inexpensive and have relatively small side-effects. Therefore, of a great importance was the discovery of the ability of various vanadium compounds to normalize many symptoms of experimental [3, 8, 11, 29] and human diabetes [1, 4, 11, 13, 17, 19, 32] e.g. elevated blood and urine sugar level, blood triglyceride and other lipid contents, as well as normalization of activity of some enzymes, participating in carbohydrate and lipid metabolism, especially phosphatases and phosphorylases [4].

Relatively limited investigations were performed on subcellular level, in association with morphological study. In the years 1997 - 2002, we studied four organic vanadium(IV) complexes and their influence on alterations of biochemical activities and morphology, characteristic for streptozotocin-diabetes rat liver Golgi complexes [9, 20 - 22].

The application in USA and Canada of some inorganic vanadium(IV) compounds, including $VOSO_4$, to ameliorate diabetic symptoms in volunteers [1, 4, 11, 13, 17, 19, 32], prompted us to investigate the action of this drug in our experimental model. The easy availability of the well-characterized vanadium compound, a relatively low cost and very simple way of application facilitated our investigations.

Material and Methods:

Animals

The experiments were carried out in four groups of female Wistar rats approximately 6 months old and weighing 200 - 250g, following the obtention of a permission of the Cracow Ethics Commission for Animal Experiments. The animals, two per a cage, were fed with standard peleted food and given tap water prior to the experiments:

1. C - the control rats that received to drink 0.09mol NaCl solution during 7 days (6 rats).
2. C+V - the rats that received to drink 3mmol $VOSO_4$ in 0.09 mol NaCl solution during 7 days (9 rats).
3. D+V - the rats in which diabetes had been induced by a single intraperitoneal injection of streptozotocin (STZ), in a dose 65mg/kg of body weight, freshly dissolved in 0.05mol citrate buffer pH 4.5. After 3 days the free blood sugar level was measured and the rats that showed a result above 250mg/100ml were given

a drinking solution of 3mmol $VOSO_4$ in 0.09mol NaCl solution during 7 days (9 rats).

4. D - the rats in which diabetes had been induced in the same way as in the D+V group, but after selection (blood sugar level above 250mg/100ml on day 3) they received 0.09mol NaCl solution for 7 days (6 rats).

In all investigated groups all rats survived the experiments. During the experiments, liquid and food consumption was measured every two days. In the last day, the animals were sacrificed, free blood sugar level was measured again, liver samples were taken for electron microscopic analysis and the remaining portion of the liver was immediately used for isolation of Golgi-rich membrane fraction followed by estimation of galactosyltransferase (GalT) activity by the Fleischer method [14].

The untreated C and D groups were studied in parallel, in identical experimental conditions (the strain and sex of animals, time and method of experiment conducing) as the vanadium treated C+V and D+V groups to allow for comparing the obtained results.

Analytical methods

Protein was estimated by the method of Lowry et al. [24] with crystalline serum bovine albumin as a standard. Free blood sugar level was estimated according to Somogyi and Nelson [30]. The Golgi-rich membrane fraction was isolated and galactosyltransferase (GalT) activity estimated according to the Fleischer [14] method.

Ultrastructural examination

For electron microscopy, four biopsy materials from each group were fixed in formaldehyde-glutaraldehyde fixative over night at 4˚C by the method of Karnovsky [18]. 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 [33]. All studies were performed under electron microscope Zeiss EM 900 operating at 80kV. EM photographs had magnification of 60 000x.

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 below in the Table 1 or Figure 1.

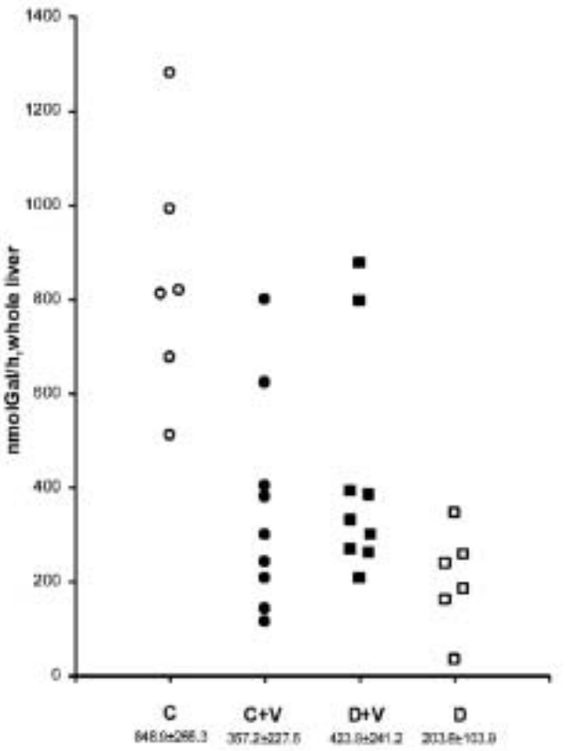


Fig. 1. Dispersion of results of estimated total galactosyltransferase activity (expressed as nmoles Gal transferred per 1 hour and per whole liver). The mean values ± SD in all study groups are given. In three groups (C+V, D+V, and D) these values are statistically significant lower than control (C) group. Particular t and p values are given below Table 1.

Reagents

Sodium cacodylate, serum bovine albumin, Folin phenol reagent, UDP-Gal, Triton X-100, streptozotocin, vanadyl sulphate, uranyl acetate, lead citrate, were obtained from Sigma-Aldrich Chemical Co., TRIS and β-mercaptoethanol came from Koch-Light Lab. Dowex 2x8 with granulation (200 - 400 mesh) came from Fluka & Buchs, ^{14}C UDP-Gal with the specific activity 292mCi/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

Table 1 summarizes some physiological and biochemical results obtained in the investigated groups. In both groups treated with vanadyl sulphate a decrease of body weight and a reduction of food intake were observed as compared to the control. In the D+V group, an improvement of polyuria, polyphagia and polydypsia was noted, however, full normal-

TABLE 1

Physiological and biochemical characteristics of study rats and isolated rat liver Golgi-rich membrane preparations

Characteristics		C (n=6)	C+V (n=9)	D+V (n=9)	D (n=6)
Body weight during experiment (g)	Start	222.5 ± 15.5	211.3 ± 15.0	221.7 ± 12.7	220.1 ± 18.4
	End	284.1 ± 58.8	202.4 ± 16.8[a)]	180.3 ± 17.5[b)]	186.2 ± 14.0
	%of changes	19.4 ± 13.3↑	4.2 ± 3.3↓	18.8 ± 4.3↓	15.3 ± 4.1↓
Liver weight (g)		9.0 ± 1.1	5.9 ± 1.0	6.0 ± 1.0	8.5 ± 1.2
Fluid intake (ml/day/rat)		27.0 ± 6.6	11.1 ± 1.4[c)]	27.3 ± 11.2[d)]	124.9 ± 12.1[e)]
Food intake (g/day/rat)		17.9 ± 1.0	12.8 ± 2.2[f)]	14.0 ± 2.9	28.1 ± 2.4[g)]
Free blood sugar level (mg/100 ml)	3-rd day after STZ	–	–	481.4 ± 141.1	396.4 ± 119.7
	Last day of experiment	126.5 ± 18.8	141.0 ± 32.9[h)]	288.8 ± 78.9[i)]	416.8 ± 132.0[j)]
% of changes in blood sugar		–	–	37.9 ± 17.9↓	4.5 ± 1.9↑
Yield of Golgi-rich fraction (mg protein/g of liver)		0.347 ± 0.122	0.239 ± 0.094	0.353 ± 0.122[k)]	0.225 ± 0.137
Specific activity of GalT (nmoles Gal/h, mg of protein)		301.0 ± 89.8	252.8 ± 116.4	213.5 ± 98.3	121.9 ± 58.3[l)]
GalT activity (nmoles Gal / h, g of liver)		93.5 ± 24.9	59.1 ± 33.0[m)]	68.7 ± 29.8	24.6 ± 13.4[n)]
Total GalT activity (nmoles Gal / h, total liver)		848.9 ± 265.3	357.6 ± 227.5[o)]	423.9 ± 241.2[p)]	203.8 ± 103.9[r)]

[a)]C/C+V: t=3.9949, 0.001<p<0.01; [b)]C/D+V: t=5.0479, p<0.001, C+V/D+V: t=2.7211, 0.01<p<0.02; [c)]C/C+V: t=7.0641, p<0.001; [d)]C+V/D+V: t=4.3200, p<0.001; [e)]D/C: t=17.3857, p<0.001, D/C+V: t=28.4793, p<0.001, D/D+V: t=16.0782, p<0.001; [f)]C/C+V: t=5 .2383, p<0.001; [g)]D/C: t=9.6467, p<0.001, D/C+V: t=12.6488, p<0.001, D/D+V: t=9.7724, p<0.001; [h)]C+V/D+V: t=5.1888, p<0.001; [i)]C/D+V: t=4.8913, p<0.001; [j)]D/C: t=5.3340 p<0.001, D/C+V: t=6.0972 p<0.001, D/D+V: t=2.3657, 0.02<p<0.05; [k)]C+V/D+V: t=2.2273, 0.02<p<0.05; [l)]D/C: t= 4.0979, 0.001<p<0.01, D/C+V: t=2.5298, 0.02<p<0.05; [m)]C/C+V: t=2.1677, p<0.05; [n)]D/C: t=5.9679, p<0.001, D/C+V: t=2.4104, 0.02<p<0.05, D/D+V: t=3.3708, 0.001<p<0.01; [o)]C/C+V: t=3.8399, 0.001<p<0.01; [p)]C/D+V: t=3.2154, 0.001<p<0.01; [r)]D/C: t=5.5458, p<0.001

ization was not achieved. In particular, polyuria was resolved as soon as after 1-day intake of vanadium solution, so far after 7 days, and blood sugar level decreased by approximately 40% in one half of diabetic animals. In comparison with untreated control (group C), the treatment of the rats in C+V group with vanadium solution caused a decrease yield of Golgi-rich membrane isolation. As compared with C group, in both groups treated with $VOSO_4$, a statistically significant decrease of galactosyltransferase (GalT) activity ($0.001<p<0.01$) was achieved (Table 1 and Fig. 1). In comparison with untreated diabetic rats (D group), in D+V group GalT activity (expressed in nmoles Gal transferred per hour and per whole liver) was elevated approximating the control level; however not quite achieving the said value. Other parameters, such as fluid and food intake, blood sugar level, as well as yield of Golgi-rich membrane fraction were also improved as compared with untreated D group. Particular t and p values are given under the Table 1.

Relatively pronounced ultrastructural changes were observed in the liver cells of the C+V group. In the majority of cases they were expressed as blurred mitochondrial structures and interrupted continuity of the nuclear envelope, leading to cellular death. Cells elimination through apoptosis was also observed. Subcellular changes were focal in character. Electron microscopy was employed in the regions of tissue with the least degree of changes. The Golgi apparatus was most frequently seen as isolated and low stacks, with a straight or undulating course. The ultimate cistern on the *trans* side was grossly distended and filled with electron-lucid material or else appeared as fragmented distended segments that were also filled with electron-lucid material similarly as numerous vacuoles, smaller or bigger in size. On the *cis* side there were noted numerous coated vesicles. In the vicinity, large multi-vesicular structures were observed, as well as double-membrane vacuoles. Large electron-dense vesicles were also encountered, similarly as double membrane vacuoles filled with electron-dense material. At sites typical for the location of Golgi structures, membranous, myelin-like structures were found.

In rats from D+V group, the ultrastructural changes were less numerous compared to the previous group. No apoptotic cells were noted. The Golgi complexes were better developed, appearing as 2 - 3 long cisterns, arched or oval in shape and distended at the margins. The external cistern was also clearly distended and filled with electron-lucid material or else it presented as fragmented, distended segments also filled with electron-lucid material, similarly as the numerous vacuoles in its vicinity. In this group the number of transport vesicles was lower. However, at a distance from the Golgi structures there were seen giant vacuoles filled with electron-lucid floccular material. In this group large, electron-dense lyzosome-like vesicles were noted.

Discussion

Vanadium derivatives - inorganic, such as ortho-, meta-vanadate or vanadyl sulphate [6, 11 - 13, 32, 36] and various organic compounds [4, 5, 11, 26 - 28, 34 - 36] manifest an

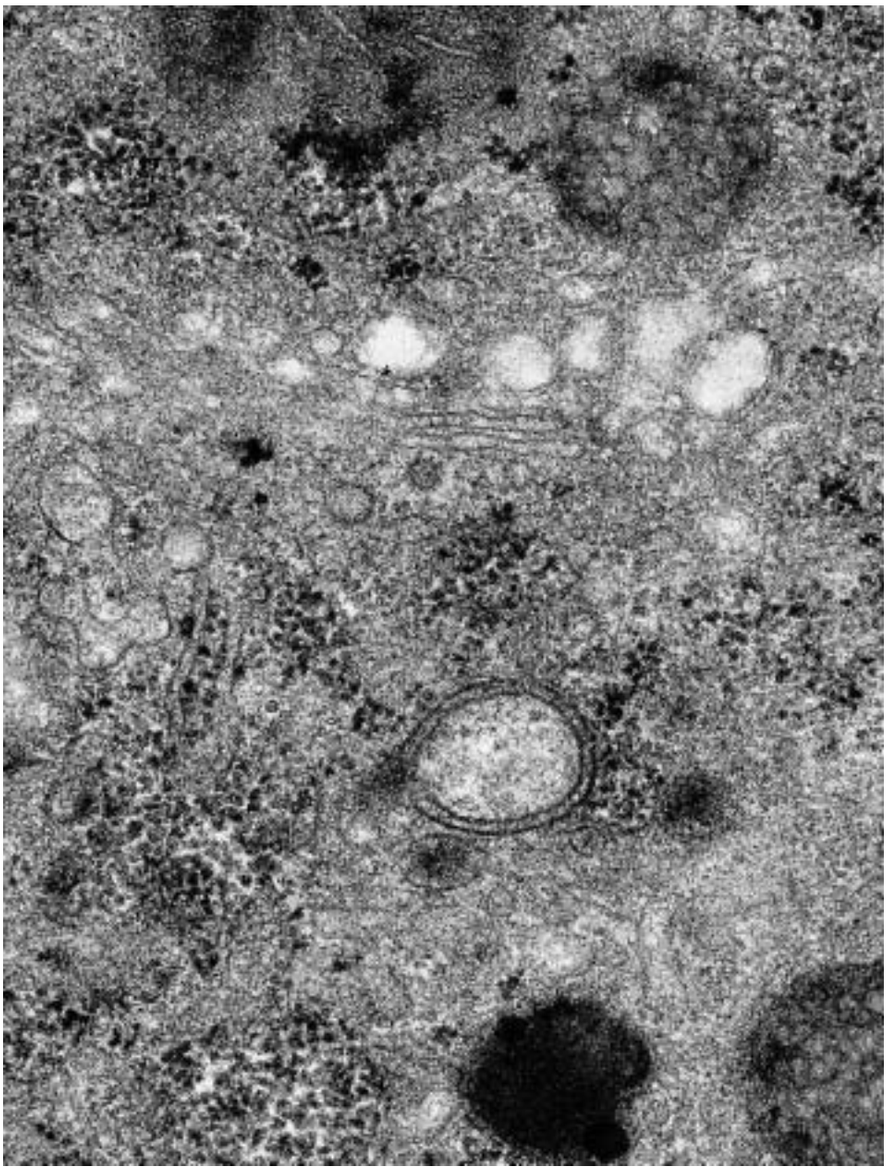


Fig. 2. C+V group. Straight Golgi stacks. The ultimate cistern on the *trans* side was grossly distended and filled with electron-lucid material or else it appeared as fragmented, distended segments. On the *cis* side there were noted numerous coated vesicles. In the vicinity, large multi-vesicular structures were observed, as well as double-membrane vacuoles. Magn. 60000x.

insulin-mimicking activity both *in vitro* and *in vivo*. They exert multiple biological effects on the whole organism [3, 25, 28, 29, 31, 37], organs, or cells in culture and interact directly with various enzymes, inhibited gluconeogenesis, glycogenolysis, lipolysis and stimulated lipogenesis [2, 7, 8, 10, 12, 13, 15, 23, 27, 28]. Recently they were used in human diabetic volunteers [1, 11, 13, 17, 19, 32]. *In vitro* investigation showed the toxicity of vanadium complexes at 1mM concentration, whereas concentration of 0.01mM and below were non-toxic; moreover vanadium as V^{+5} is less toxic than V^{+4} [27]. Contrary to these data, the *in vivo* study showed that vanadyl ion (V^{+4}) was less toxic then meta- or orthovanadate [4, 11, 36]. Metavanadate was applicated in a drinking solution (0.15mg/ml) for 28 days or 1.1mg/ml for 2 weeks. In contrast, no evidence of toxicity was reported when vanadyl sulphate was given at the dose of 0.5 - 1.5mg/ml for one year to STZ-diabetic rats [11]. It was one of the reasons to use $VOSO_4$ as an anti-diabetic drug in humans. The effectiveness of vanadium therapy depends *in vivo* on the type of vanadium ligands, the dose, treatment duration and modality, the species of animal used, as well as the clinical status of treated animals [4, 11]. Generally, organic compounds are used in lower doses than inorganic vanadium complexes, such as VO_4^{3-} and VO_3^{1-} or VO^{2+}, and with a better biological effect [36]. Our previous investigations have demonstrated, that four organic vanadium derivatives exerted a diversified effect over a short time acting as normalizing "drugs" on changes in rat liver Golgi complexes [9, 20 - 22]. In our model of experiment, the effectiveness of these compound may be listed as follows: bis(maltolato)oxovanadium [BMOV] > bis(2,2'-bipyridine)oxovanadium [$VO(bpy)_2$] > bis(oxalato)oxovanadium > bis(kojato)oxo-

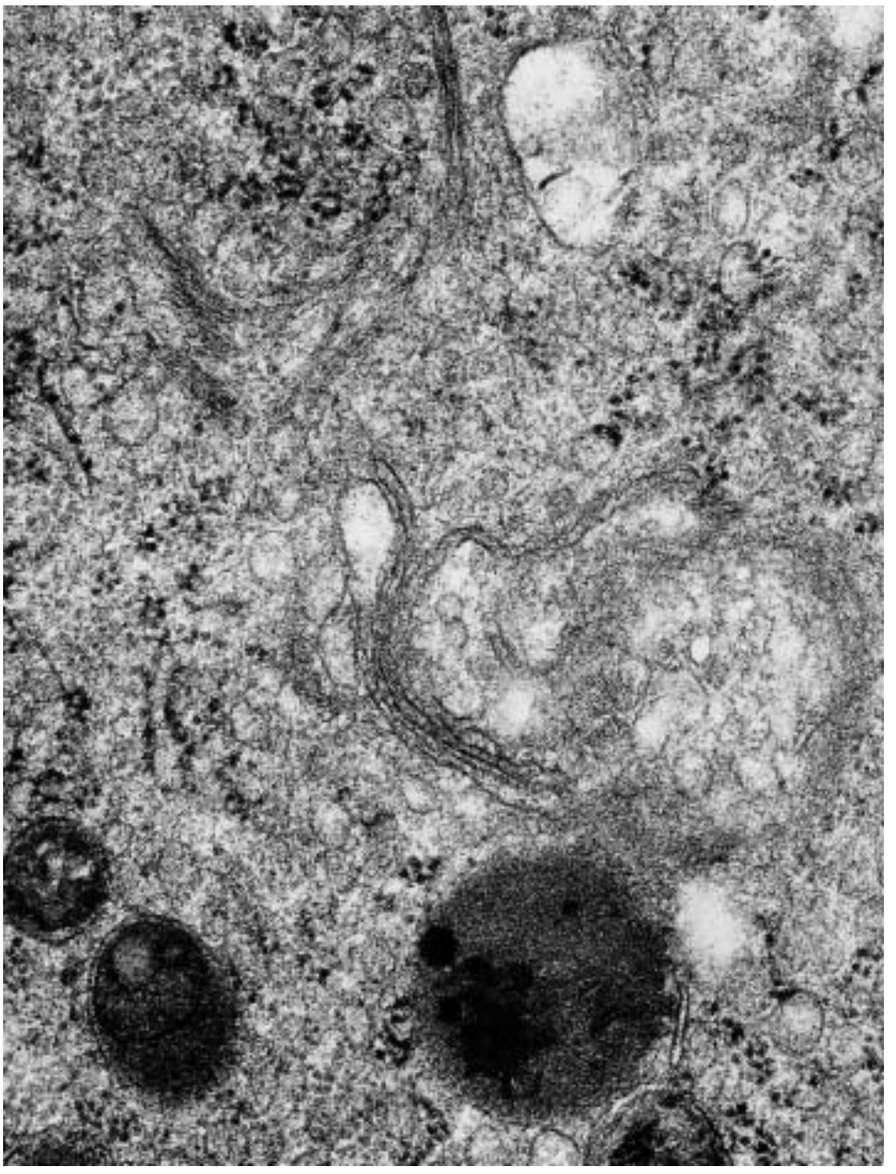


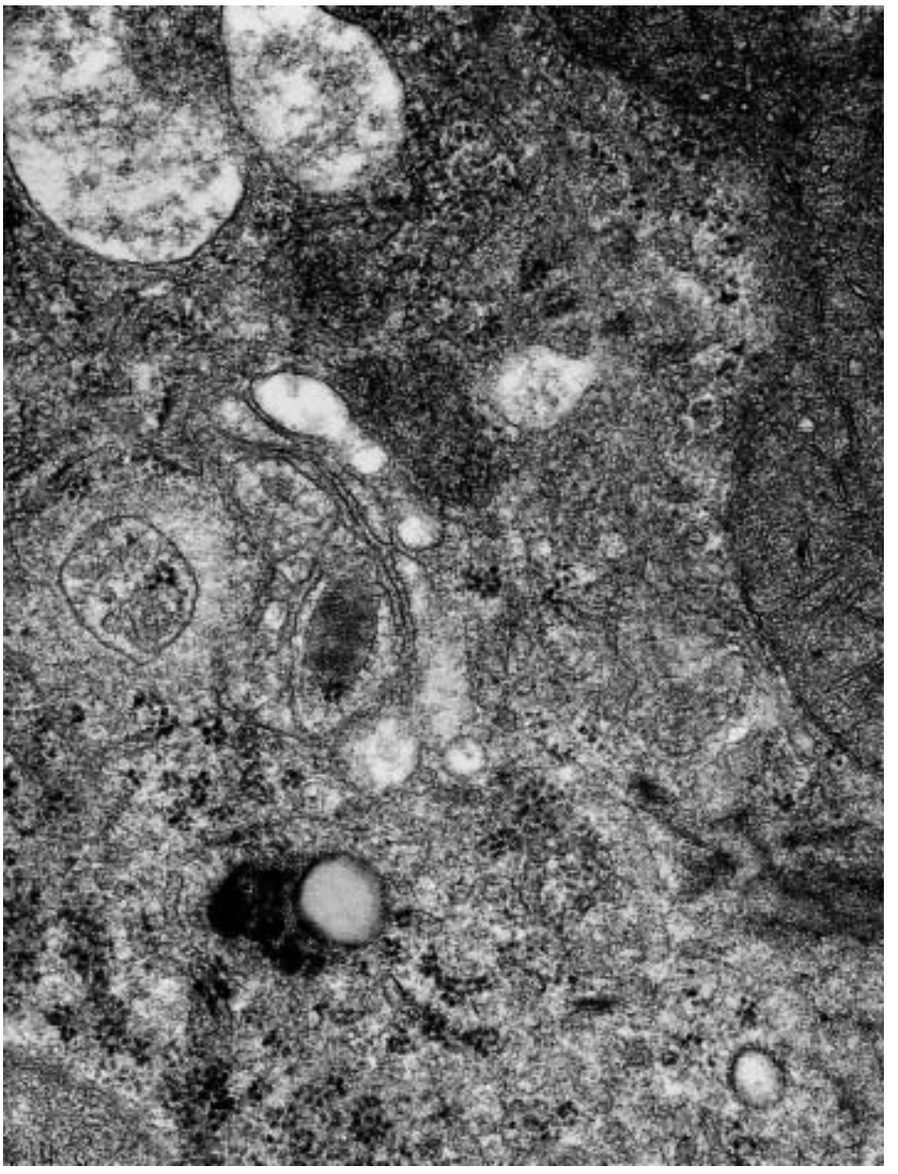
Fig. 3. D+V group. The Golgi complexes appear as 2 - 3 long cisterns, arched or oval in shape and distended at the margins. In this group large, electron-dense lyzosome-like vesicles were noted. Magn. 60000x.

vanadium [$VO(ka)_2$]. It is interesting to note, that the first and last compounds are chemical analogues [35].

This paper describes influence of $VOSO_4$ on the biochemical function and morphology of rat liver Golgi complexes. In our experiment, vanadyl sulphate concentration as 0.75mg/ml in 0.5% NaCl as drinking fluid for 7 days was used. It was on the down limit listed above values [11]. In both the vanadium-treated groups, all the animals survived. It is especially important in the D+V group, which received two drugs with a strong biological activity. All vanadium solutions are willingly consumed by rats, and dehydration, especially in diabetic rats, is a life-threatening condition. In our D+V group, the liquid intake was the same as in C group (where the rats received only 0.5% NaCl), but in the C+V group, intake was statistically lower than in the C group.

In both vanadium-treated groups activity of galactosyltransferase (GalT) - the liver Golgi complexes marker enzyme - was lower than in the control group. Although in the C+V group the activity expressed per g of liver or per whole liver was statistically significant ($p<0.05$ or $p<0.01$), in the D+V group we observed an increase of GalT activity, which approximated the control value; however, the total enzyme activity was lower than in the control ($p<0.01$). Additionally, the yield of Golgi-rich membrane isolate was normalized to the control level in the D+V group.

Ultrastructural changes of Golgi complexes described by Ghadially showed their sensitivity (morphological lability) to the cell activity [16]. The author described destructive changes in Golgi complexes or atrophy of some of its elements in hepatocytes exposed to various toxic agents [16].



A

Fig. 4A (page 30) and B (page 31). D+V group. The number of transport vesicles was lower. However, at a distance from the Golgi structures there were seen giant vacuoles filled with electron-lucid floccular material. Magn. 60000x

In our morphological investigations we have demonstrated significant alterations of hepatocyte structure with a relatively small destruction of the region of Golgi complexes in the control, vanadyl treated rats. In STZ-diabetic, treated with vanadium rat livers, relatively small changes of cell structure, but greater alterations in the region of Golgi complexes were observed. In our opinion $VOSO_4$, applied in lower doses as a drinking solution over a relatively short time, caused greater changes in the liver cells of control as compared to the STZ-diabetic rats, in which acted as a normalizing drug.

Acknowledgements: The authors are grateful to Sławomir Kordowiak Jr, for his computer processing of the results and preparing the graph in Fig. 1.

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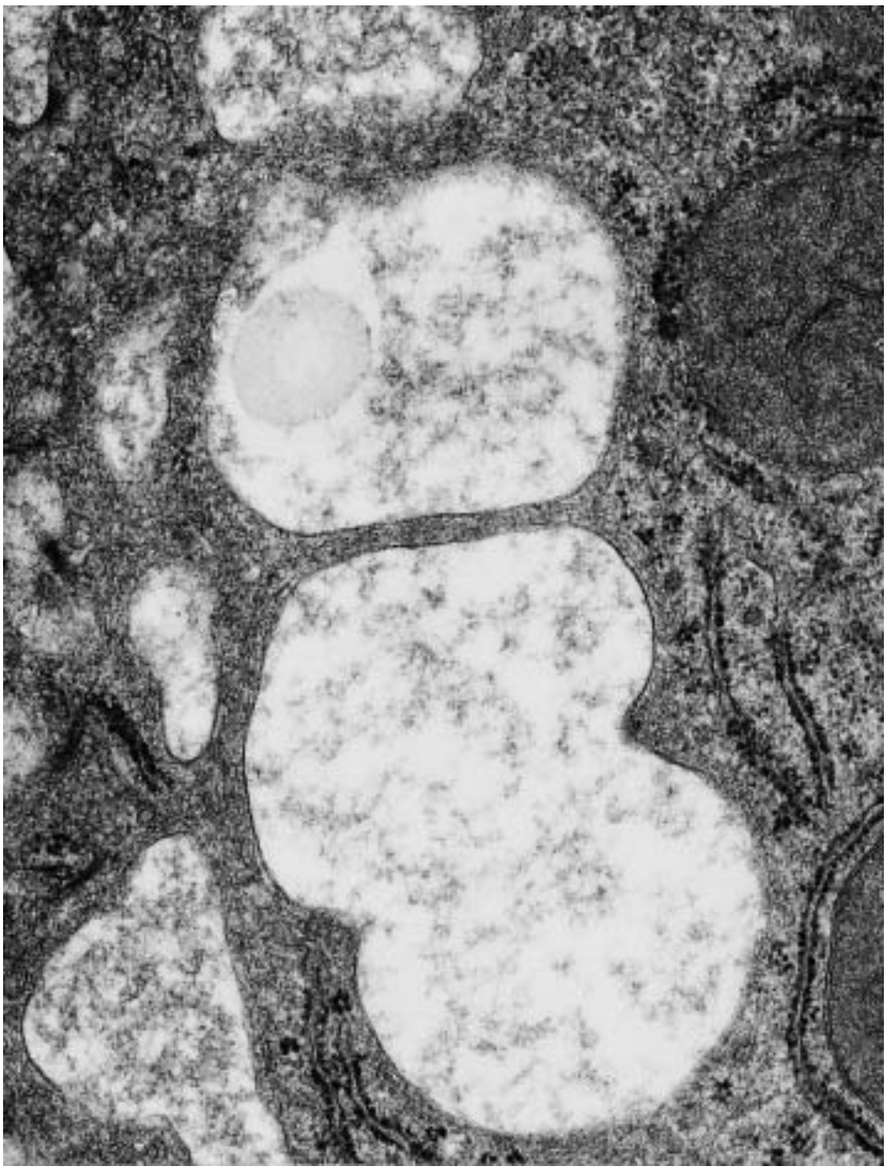

B

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