The authors describe the influence of vanadyl sulphate on liver Golgi complexes in control and streptozotocin (STZ)-diabetic rats. VO
SO$_4$, 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 vanadylions, although STZ-diabetic, vanadium treated rats were affected by two types of adverse effects exerted 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.

The application in USA and Canada of some inorganic vanadium(IV) compounds, including VO
SO$_4$, 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 pellet 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 VO
SO$_4$  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
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 conducting) 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.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>C (n=6)</th>
<th>C+V (n=9)</th>
<th>D+V (n=9)</th>
<th>D (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight during experiment (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>222.5 ± 15.5</td>
<td>211.3 ± 15.0</td>
<td>221.7 ± 12.7</td>
<td>220.1 ± 18.4</td>
</tr>
<tr>
<td>End</td>
<td>284.1 ± 58.8</td>
<td>202.4 ± 16.8</td>
<td>180.3 ± 17.5</td>
<td>186.2 ± 14.0</td>
</tr>
<tr>
<td>% of changes</td>
<td>19.4 ± 13.3 1</td>
<td>4.2 ± 3.3 4</td>
<td>18.8 ± 4.3 4</td>
<td>15.3 ± 4.1 4</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>9.0 ± 1.1</td>
<td>5.9 ± 1.0</td>
<td>6.0 ± 1.0</td>
<td>8.5 ± 1.2</td>
</tr>
<tr>
<td>Fluid intake (ml/day/rat)</td>
<td>27.0 ± 6.6</td>
<td>11.1 ± 1.4 2</td>
<td>27.3 ± 11.2 2</td>
<td>124.9 ± 12.1 2</td>
</tr>
<tr>
<td>Food intake (g/day/rat)</td>
<td>17.9 ± 1.0</td>
<td>12.8 ± 2.2 1</td>
<td>14.0 ± 2.9</td>
<td>28.1 ± 2.4 3</td>
</tr>
<tr>
<td>Free blood sugar level (mg/100 ml)</td>
<td>3-rd day after STZ</td>
<td>–</td>
<td>–</td>
<td>481.4 ± 141.1</td>
</tr>
<tr>
<td>Last day of experiment</td>
<td>126.5 ± 18.1</td>
<td>140.0 ± 32.9 3</td>
<td>288.8 ± 78.9 3</td>
<td>416.8 ± 132.0 3</td>
</tr>
<tr>
<td>% of changes in blood sugar</td>
<td>–</td>
<td>–</td>
<td>37.9 ± 17.9 4</td>
<td>4.5 ± 1.9 7</td>
</tr>
<tr>
<td>Yield of Golgi-rich fraction (mg protein/g of liver)</td>
<td>0.347 ± 0.122</td>
<td>0.239 ± 0.094</td>
<td>0.353 ± 0.122 8</td>
<td>0.225 ± 0.137 8</td>
</tr>
<tr>
<td>Specific activity of GalT (nmoles Gal/h, mg of protein)</td>
<td>301.0 ± 89.8</td>
<td>252.8 ± 116.4</td>
<td>213.5 ± 98.3</td>
<td>121.9 ± 58.3 2</td>
</tr>
<tr>
<td>GalT activity (nmoles Gal/h, g of liver)</td>
<td>93.5 ± 24.9</td>
<td>59.1 ± 33.0 3</td>
<td>68.7 ± 29.8</td>
<td>24.6 ± 13.4 3</td>
</tr>
<tr>
<td>Total GalT activity (nmoles Gal / h, total liver)</td>
<td>848.9 ± 265.3</td>
<td>357.6 ± 227.5 3</td>
<td>423.9 ± 241.2 3</td>
<td>203.8 ± 103.9 3</td>
</tr>
</tbody>
</table>

4/5/C/V: t=3.9949, 0.001 < p < 0.01; $^{10}$/C/+/V: t=5.0479, p=0.001; C+/+V/+: t=2.7211, 0.01 < p < 0.02; $^{10}$/C/C+/V: t=7.0641, p<0.001; $^{10}$/C+/V/+: t=3.4200, p<0.001; $^{10}$/D/C+: t=17.3857, p<0.001; $^{10}$/D+/+V/+: t=28.4793, p<0.001; $^{10}$/D+/V/+: t=16.7082, p<0.001; $^{10}$/C/C+: t=2.8383, p<0.001; $^{10}$/D/C+: t=9.6467, p<0.001; $^{10}$/D/C+: t=12.6488, p<0.001; $^{10}$/D+/+V/+: t=9.7724, p<0.001; $^{10}$/C+/+V/+: t=5.1888, p<0.001; $^{10}$/D+/V/+: t=4.8913, p<0.001; $^{10}$/D:C/+: t=5.3340, p<0.001; $^{10}$/D+C+: t=6.0972, p<0.001; $^{10}$/D+/V+: t=2.3657, 0.02 < p < 0.05; $^{10}$/C+/+V/+: t=2.2723, 0.02 < p < 0.05; $^{10}$/D+/V/+: t=4.0979, 0.001 < p < 0.01; $^{10}$/D/C+: t=2.5298, 0.02 < p < 0.05; $^{10}$/C+C+: t=2.1677, p<0.05; $^{10}$/D:C/+: t=5.9679, p<0.001; $^{10}$/D+C+: t=2.4104, 0.02 < p < 0.05; $^{10}$/D+/V/+: t=3.3708, 0.001 < p < 0.01; $^{10}$/C+C+: t=3.4390, 0.001 < p < 0.01; $^{10}$/D+C+: t=3.2154, p<0.001; $^{10}$/D/C+: t=5.5458, p<0.001

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

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
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, 31]. 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 than metavanadate [4, 11, 36]. Metavanadate was applied 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. 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.
vanadium [VO(ka)₂]. It is interesting to note, that the first
and last compounds are chemical analogues [35].

This paper describes influence of VO₄ on the bio-
chemical function and morphology of rat liver Golgi com-
plexes. 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 galacto-
syltransferase (GaT) - 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
GaT 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 la-
bility) to the cell activity [16]. The author described destruc-
tive changes in Golgi complexes or atrophy of some of its
elements in hepatocytes exposed to various toxic agents [16].
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 VOSO₄, 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.

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References