MODIFICATIONS OF THE PROTEINS, LIPOPROTEIDS AND GLUCOPROTEIDS IN THE BLOOD OF PATIENTS SUFFERING FROM DIABETES MELLITUS

By M. Sheykman

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MODIFICATIONS OF THE PROTEINS, LIPOPROTEIDS, AND GLUCOPROTEIDS IN THE BLOOD OF PATIENTS SUFFERING FROM DIABETES MELLITUS

Following is the translation of an article by M. B. Sheykm (Moscow) entitled "Izmeneniya Belkov, Lipoproteidov i Glyukoproteidov Krovi v Bol'nykh Sakharnym Diabetom" (English version above) in Klinicheskaya Meditsina (Clinical Medicine), Vol. 39, No. 7, July 60, pages 36-43.

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The physiological importance of the simple and complex proteins and proteids of plasma is very great. The successes achieved in recent years in the physicochemical study of proteins (by electrophoresis, ultracentrifugation, etc.) has made it possible to undertake a more thorough study of the protein-proteid composition of the blood under various pathological conditions. The accumulated observations have made it possible to speak of blood protein changes characteristic of various diseases, and have aided substantially in the physiotopathological and clinical characterization of some of them. The changes in proteins and proteids (lipoproteids and glucoproteids) of the blood plasma, which depend on the character and degree of impairment of metabolic processes, have definite diagnostic and differential-diagnostic and great prognostic value in a number of diseases (Antweiler, Wuhrman and Wunderly, Riva, Emmrich, Girard).

In diabetes mellitus considerable modification occurs in the metabolism of simple and complex proteins, as a result of impairment of the neuroendocrine regulation of metabolic processes. A change in the protein-proteid blood composition is one of the manifestations of these metabolic disturbances. The origin and character of these changes have not yet been
studied sufficiently. The deficit of insulin and of the lipotropic factor of the pancreas, as well as the impaired functioning of the hypophysis, suprarenal and genital glands, are important factors in bringing about these changes. It has been elicited experimentally that insulin doses which contribute to protein synthesis exceed three to five fold times the amount of insulin needed for the maintenance of normal glycemia (Wenderoth). Considerable modifications of the protein-proteid composition of blood were elicited in experimental diabetes (Lewis, Cristiansson, Peterson and Beatty), in hypophysectomy, adrenalectomy, and administration of sexual hormones as well as of hormones of the anterior hypophyseal lobe and the adrenal cortex (Bernasconi, Del Vecchio, et al., Aschkenasy, Furman and Howard, Berezin and Studnitz, bellien). In recent years there have appeared in the foreign literature a number of works devoted to the study of the protein-proteid blood composition in patients with diabetes mellitus. The basic changes in blood serum proteins in diabetic patients were manifested in the form of hypoalbuminemia and hyperglobulinemia. Different changes in the globulin fractions were elicited (the greatest changes were elicited by various authors in alpha-globulin, in beta-globulin, simultaneously in alpha2- and beta-globulins or in gamma-globulin), which resulted in contradictory data in the literature (Beckman, Bogdanovich, et al; Ralli, et al; Scheurlen, de-Petra and Frinci; Melingoff, et al; Lewis, et al; Lederer, et al; Schertenleib, et al; Introzzi, et al.). There is no unanimous opinion as to the character of the changes of blood lipoproteids in diabetes. The majority of authors noted in diabetic patients, especially in the presence of atherosclerosis, the predominance in the blood of beta-lipoproteid richer in cholesterin and more labile (lipoproteid of the Sf-10-20 Hoffman ultracentrifugation class; Baker, et al.; Lowy and Barach; Ferguglio and Rimini; Polosa and Motta; Azerad, et al; Tolomelli, et al.; Weicker and Ley; Kuguda, et al; Vannini, et al.). A number of authors noted a considerably increased number of glucoproteids in the blood of diabetic patients (hexoses bound with protein, glucosamine, etc.), especially in the alpha2- fraction. These changes depend on the duration of the diabetes and the degree of degenerative changes in the blood vessels (Mohnike, Jannert, et al.; Bergstermann, Jacobs, Galletti, et al; Jilililand, et al; Nielsen and Poulsen; Lerman, et al.; Andriani, et al; Bastoni, et al.; Candor, Nervi and de Petra; Orabona, et al.). Taking into account the experimental data, which attest to the indubitable pathogenic significance of the impairment of the metabolism of simple and complex proteins (lipoproteids
and glucoproteids) in the development of atherosclerosis (works of N. N. Anichkov and his school; Antonini and Salvini, et al.), a number of authors contend that there exist predisposing conditions in the organisms of a diabetic patient for comparatively early and frequent development of atherosclerosis. Dickenson, as well as Wenderoth and other authors, relate the development of vascular complications in diabetes, including atherosclerosis, to a chronic protein deficiency of the organism resulting from increased neoglucogenesis. N. N. Anichkov attributes considerable importance in the development of vascular complications in diabetes to dyslipoproteidemia and hypercholesterinemia; at the same time Mohnike, as well as Introzzi, attatches considerable importance to the disturbance of glucoproteid metabolism. We must note that in atherosclerotic patients without diabetes, the changes in the blood protein composition are similar, to a great extent, to the changes observed in diabetes mellitus (M. V. Bavina, V. M. Baytsev, P. Ye. Lukomskiy, M. M. Pokhno, Antonini and Salvini, Raynaud, et al., Foigg and Schrader, Barr, Rass, et al.).

The problems of the pathogenesis of vascular complications in diabetic patients, as well as the problems of pathogenesis of atherosclerosis, are incompletely clarified and require further thorough study. Apparently, in the development of the vascular complications of diabetes, atherosclerosis in particular, a number of factors are of pathogenic significance, in particular the metabolic disorders which develop in diabetes. Of undoubted pathogenic significance are the metabolic disturbances which induce changes in the protein-proteid blood composition and hypercholesterinemia. The vascular changes in diabetic patients are the results of complex metabolic disturbances in the vascular walls which are infiltrated by protein-lipid and protein-polysaccharide components of plasma (N. N. Anichkov, Mohnike, Antonini and Salvini).

Degenerative vascular changes, which are observed in nearly 70 percent of the diabetic patients, are considered at present to be the basic cause of death for diabetics (V. M. Kogan-Yasnyy). The statistics of recent years point to the high and constantly increasing percentage of mortality of diabetic patients from vascular causes and a corresponding reduction of the rate of mortality from diabetic coma (V. M. Kogan-Yasnyy, Joslin, Warren and Le Compte, Danovskiy, Kaeding, Boller). The diabetic patients die, according to the data of pathological-anatomical studies carried out during the past 20-25 years, mostly from the impairment of circulation in the cardiac blood vessels, kidneys, brain and lower
extremities (V. M. Kogan-Yasnyy, Joslin, Boller). Atherosclerosis develops in diabetes mellitus patients at an earlier age, and a number of authors connect the frequency and gravity of atherosclerosis in these patients with the duration of the diabetic condition (N. N. Anichkov, V. M. Kogan-Yasnyy, Matthews, Paskhls, et al., Bryfogle and Bradley); this statement has been disputed by some authors (Boas, Moschovitz, E. G. Moscovich). Skoudy and other researchers think that an adequate therapy started promptly is the basic factor in preventing early development of vascular complications in diabetic patients.

In certain clinical forms of diabetes mellitus a good therapeutic effect was obtained with oral administration of hypoglycemic sulfanilamide preparations such as nadisen, rastinon, etc. (Bertram, et al., Boulin, Bender, et al., N. A. Shereshes任y). At present the mechanism and character of effect of these preparations on the metabolic processes of the organism still need to be fully clarified. Kaasch observed impairment of fat metabolism and development of obesity in patients who had been given sulfanilamide preparations (S. M. Leytes). There are contradictory data in the work which has appeared during the past two to three years in the foreign literature on the effect of sulfanilamide preparations on the protein-proteid composition of the blood of diabetic patients: some authors note aggravation of dysproteinemia while others do not corroborate this conclusion (Chatagnon and Chatagnon, Kaeding, Muting, Kuhne, Galletti, et al., V Vanini, et al.).

Taking into account the presence of contradictory data in the foreign literature, the absence of analogous studies in the Soviet literature, and the great scientific and practical importance of this problem, we carried out an investigation of the protein-proteid composition of the blood serum in 114 diabetic patients between the ages of 17 and 76 years, who had had the disease from one or two months -- up to 33 years, without or with atherosclerotic phenomena, and who received insulin therapy and sulfanilamide preparations. Dynamic studies were carried out on 88 patients. Blood for examination was obtained in the morning on an empty stomach. Total protein was determined by the refractometric method. Fractionation of proteins and proteids of the blood serum was carried out by paper electrophoresis. The electrophoregrams were stained for protein fractions with an acid blue-black dye for lipoproteids with sudan black "B," and those for glucoproteids with the Schiff reagent (according to Rushkovsky). Hexose, bound with the serum proteins, was determined by the method of Jannell et al. The data on the protein-
proteid composition of the blood, obtained by us, were compared with the clinical picture, with the dynamics of the disease, as well as with the results of other studies. The control group consisted of 59 normally healthy individuals between 20 and 50 years old.

The study of the electrophoreograms of the healthy individuals enabled us to establish the following average norms of the content of protein lipoprotein and glucoproteid fractions in the blood serum: albumins, 60.25%; globulin fractions: alpha₁, 4.03%; alpha₂, 7.9%; beta 70.95%; glucoproteids: albumins + alpha₁, 23.20%; alpha₂, 36.64%; beta 28.95%; gamma 11.21% (Table 1). The total blood serum protein was 7.58 gm-% (6.9-8.04 gm-%); hexoses bound with blood serum proteins were 113 mg-% (86-139 mg-%). The average figures for the contents of proteins and proteids of the blood serum in healthy individuals, as obtained by us, coincide with the data in the literature (Bogdanovich, et al., Scheurlen, Grassman and Hanning, Colve, P. Ye. Lukomskiy, Rushkovskiy, et al.).

The diabetic patients were divided into two groups. The first group was composed of 36 patients with uncomplicated diabetes mellitus, (i.e., patients who had given no clinical evidence of atherosclerosis on examination). The other group consisted of 78 diabetic patients with definite clinical symptoms of atherosclerosis of the aorta, cardiac vessels, brain, eye fundus, and lower extremities, which were confirmed via electrocardiographic and oscillographic examinations and ophtalmoscopy of the eye fundus. The second group of patients, which included diabetics and atherosclerotics, was characterized by a higher average age and longer duration of the disease as compared to the first group. In view of the difficulty of determining accurately in a number of cases the sequence of development of diabetes and atherosclerosis, and in view of the mention in the literature of the possibility of an independent development of these two diseases (Boas; Moschowitz; E. G. Moskovich), we found it in expedient, for lack of a sufficiently reliable criterion, to divide the patients into subgroups of cases of atherosclerosis as a complication of diabetes or as a concomitant disease. Patients who during the investigation manifested other diseases accompanied by changes in the protein-proteid composition of the blood serum (infectious diseases, diseases of the thyroid, malignant tumors, etc.) were not included in this study. The studies were conducted under clinical conditions. Dynamic study of the protein-proteid blood composition was carried out on 88 patients (from two to nine times on each patient); in some patients the protein composition of the blood was investigated upon the patients second admission.
Table 1
Protein, lipoprotein and glucoprotein fractions of blood serum in healthy individuals, uncomplicated diabetic patients, and diabetic patients with atherosclerosis (mean indices).

<table>
<thead>
<tr>
<th>Protein-proteins</th>
<th>Healthy Individuals</th>
<th>Patients with uncomplicated diabetes mellitus</th>
<th>Patients with diabetes and atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$ \mu \pm \sigma$</td>
<td>$M \pm \sigma$</td>
<td>$M \pm \sigma$</td>
</tr>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumins</td>
<td>60.25±4.14</td>
<td>56.11±3.46</td>
<td>47±3.18</td>
</tr>
<tr>
<td>Globulins: alpha</td>
<td>4.03±1.09</td>
<td>3.44±0.84</td>
<td>3.8±0.63</td>
</tr>
<tr>
<td>&quot; alpha$_2$</td>
<td>7.90±1.38</td>
<td>10.7 ±2.15</td>
<td>11.48±1.64</td>
</tr>
<tr>
<td>&quot; beta</td>
<td>11.81±1.44</td>
<td>12.85±1.7</td>
<td>16.91±2.82</td>
</tr>
<tr>
<td>&quot; gamma</td>
<td>16.01±2.05</td>
<td>16.9 ±1.97</td>
<td>20.81±2.14</td>
</tr>
<tr>
<td>Lipoproteins:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha</td>
<td>29.05±2.81</td>
<td>197.84±38</td>
<td>15.11±8.2</td>
</tr>
<tr>
<td>beta</td>
<td>70.95±2.81</td>
<td>80.16±7.38</td>
<td>84.89±8.2</td>
</tr>
<tr>
<td>Glucoproteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumins + alpha</td>
<td>23.2±3.16</td>
<td>11.41±2.97</td>
<td>8.62±2.51</td>
</tr>
<tr>
<td>alpha$_2$</td>
<td>36.64±4.92</td>
<td>49.83±5.04</td>
<td>58.52±4.74</td>
</tr>
<tr>
<td>&quot; beta</td>
<td>28.95±3.46</td>
<td>30.25±4.62</td>
<td>27±3.17</td>
</tr>
<tr>
<td>&quot; gamma</td>
<td>11.21±2.94</td>
<td>8.51±2.56</td>
<td>5.86±1.97</td>
</tr>
</tbody>
</table>

(1) $\mu$ - arithmetical mean; $\sigma$ - r.m.s. deviation.
to the clinic, six to 17 months after the first study.

In comparing the data on the protein-proteid blood composition in healthy individuals and in diabetic patients, one can observe in the latter the presence of dysproteinemia, dyslipoproteidemia and dysglucoproteidemia. These changes are more pronounced in patients suffering from diabetes and atherosclerosis. In analyzing the results of studies carried out on 36 diabetic patients with no atherosclerosis, one can observe an increase in alpha\textsubscript{2}-globulin reaching in some cases 12.8 to 13.6%, an increase in beta-lipoproteins of at most 91-94.6%, and a marked increase in alpha\textsubscript{2}-glucoproteid (up to 57-60.5%) in several patients. The mean content of hexoses bound with proteins was 127 mg-%; the highest contents in this group of patients were 149 to 152 mg-%. The total blood cholesterin (according to Blur) in patients of this group exceeded normal figures, reaching 300 mg-%; the mean figure for this group was 214 mg-%.

Daily, for three to six days, the protein-proteid blood content of 14 patients of this group was measured by paper electrophoresis and the data showed that changes in the proteins, lipoproteids and glucoproteids of the blood serum had no constant character. During the period of investigation fluctuations were observed in the protein-proteid blood composition of the patients. Most constant was the increase in alpha\textsubscript{2}-globulin and alpha\textsubscript{2}-glucoproteid. A correlation was observed between the seriousness of the diabetic condition and the protein-proteid composition of the blood. In comparing the results of studies on patients of this group, who varied as to the seriousness of their condition, we note considerably greater deviations from normal in patients in whom the disease had progressed further. Thus, in seven patients of this group with comparatively mild diabetes, during the period of compensation the content of albumin averaged 59.2%, alpha\textsubscript{2}-globulin 8.3%, beta-lipoproteids 76.9%, and alpha\textsubscript{2}-glucoproteids 44.1%, whereas in eight patients of the same group with more severe diabetes (higher glycemia, glucosuria, acetonuria) the mean content of albumin 51.3%, alpha-globulin 10.8%, beta-lipoproteid 51.8%, alpha\textsubscript{2}-glucoproteid 55.6%. These data indicate the presence of more serious disturbances of protein metabolism in patients with a more severe diabetes.

In diabetic patients with marked atherosclerotic manifestations there are observed more pronounced dysproteinemia, dyslipoproteidemia and dysglucoproteinemia than in patients with simple diabetes (see Table 1). Hypoalbuminemia, more pronounced in patients of this group, reached 34 to 37.4% in some cases. The globulin content was increased, mainly in the
beta and alpha\textsubscript{2} fractions. The percentage of beta-lipoproteid was considerably increased, reaching in some patients 93.5\%. Dysglucoproteidemia was more pronounced. The content of hexoses bound with proteins in some patients reached 200 mg-% (168 mg-% on the average), and hypercholesterinemia ranged up to 380 to 450 mg-% (249 mg-% on the average).

Daily determinations of the protein-proteid content of the blood by paper electrophoresis, conducted on 31 patients of the second group for a period of three to six days, showed individual changes more stable than the changes in patients of the first group. The most constant changes, which are characteristic for patients suffering from diabetes with atherosclerosis, are increases in beta-globulin, beta-lipoproteid and alpha\textsubscript{2}-glucoproteid. The protein-proteid changes in the blood depend on the severity of the atherosclerotic changes in the patients. Thus, in 14 patients with a mild form of atherosclerosis the mean contents were: albumin 50.1\%, beta-globulin 12.8\%, beta-lipoproteid 51.7\%, alpha\textsubscript{2}-glucoproteid 51.6\%, and hexoses bound with serum proteins 143 mg\%. In 12 patients with atherosclerotic vascular changes that were clinically more pronounced (patients who had a history of a myocardial infarct, or cerebral hemorrhage, or marked vascular changes in the lower extremities), the average quantities were: albumin 44.2\%, beta-globulin 17.3\%, beta-lipoproteid 66.2\%, alpha\textsubscript{2}-glucoproteid 58.5\%, hexoses bound to protein 189 mg\%. The content of total blood serum proteins in both groups varied within normal limits (the average figure for the first group was 7.25 gm-%, that for the second, 7.41 gm-%).

Dynamic studies of the protein-proteid composition of blood were carried out on 47 patients treated with insulin, and on 41 patients treated with sulfanilamide preparations. The insulin therapy was carried out on 17 diabetic patients who had no atherosclerosis, and on 30 patients with clinically pronounced atherosclerosis. Sulfanilamide preparations were administered to 10 nonatherosclerotic patients and to 31 diabetic and atherosclerotic patients. In patients with uncomplicated diabetes who had been on a limited carbohydrate diet with insulin therapy as well as with sulfanilamide treatment there occurred definite normalization of the blood protein composition (Table 2). Normalization of the protein formula in patients with uncomplicated diabetes with insulin therapy was observed in seven cases, while with sulfanilamide treatments normalization occurred in three cases; discernible changes in the blood protein composition with a higher or lower approximation to normal figures (increase in albumin content, decrease in alpha\textsubscript{2}-globulin, beta-lipoproteid and alpha\textsubscript{2}-glucoproteid levels) -- correspondingly in
### Table 2

Content of separate fractions of lipoproteids and glucoproteids of the blood serum of diabetic patients treated with insulin and sulfanilamide preparations

<table>
<thead>
<tr>
<th>Protein-proteids</th>
<th>Patients treated with insulin</th>
<th>Diabetes without atherosclerosis</th>
<th>Diabetes with atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At Admission</td>
<td>At discharge</td>
<td>At Admission</td>
</tr>
<tr>
<td></td>
<td>$M \pm \sigma$</td>
<td>$M \pm \sigma$</td>
<td>$M \pm \sigma$</td>
</tr>
<tr>
<td>Albumin</td>
<td>56.27±3.11</td>
<td>60.16±2.87</td>
<td>50.33±4.72</td>
</tr>
<tr>
<td>alpha₂-globulin</td>
<td>9.7±2.17</td>
<td>8.04±1.29</td>
<td>10.8±1.38</td>
</tr>
<tr>
<td>beta-globulin</td>
<td>12±1.79</td>
<td>11.25±2.04</td>
<td>15.17±1.38</td>
</tr>
<tr>
<td>beta-lipoproteid</td>
<td>80.45±6.54</td>
<td>74.34±5.3</td>
<td>83.5±7.06</td>
</tr>
<tr>
<td>alpha₂-gluco-proteid</td>
<td>45.17±5.38</td>
<td>40.05±6</td>
<td>53.65±4.71</td>
</tr>
</tbody>
</table>

(Table 2 continued on next page)
<table>
<thead>
<tr>
<th>Patients treated with sulfanilamide preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes without atherosclerosis</td>
</tr>
<tr>
<td>At admission</td>
</tr>
<tr>
<td>M ± σ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein Proteins</th>
<th>Content of protein proteids in percent (average figures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>58.1 ± 2.54</td>
</tr>
<tr>
<td>alpha&lt;sub&gt;2&lt;/sub&gt;-globulin</td>
<td>9.56 ± 2.05</td>
</tr>
<tr>
<td>beta-globulin</td>
<td>11.25 ± 2.96</td>
</tr>
<tr>
<td>beta-lipoproteid</td>
<td>79.09 ± 8.22</td>
</tr>
<tr>
<td>alpha&lt;sub&gt;2&lt;/sub&gt;-glucoproteid</td>
<td>43.81 ± 4.78</td>
</tr>
</tbody>
</table>
eight out of six patient $\sqrt{5}c^7$. The protein-proteid composition of the blood remained unchanged in two discharged patients treated with insulin, and in one patient who was taking nadisan. Good therapeutic effects upon administration of insulin or sulfanilamide preparations (decrease in glucemia and glucosuria, disappearance of acetonuria, and improvement of the general condition were accompanied, as a rule, by greater of lesser normalization of protein blood indices.

In diabetic patients with clinically marked atherosclerosis, normalization of protein-proteid composition of the blood in insulin therapy was noted in four cases only; approximation of protein blood indices to normal figures occurred in 15 cases; no changes were noted in 11 cases. In patients who had been given sulfanilamide preparations, normalization of protein blood composition was not observed in a single patient, approximate normalization was observed in 12 patients; no change in the protein-proteid composition of the blood was found in 19 patients. Lack of normalization of the protein-proteid blood composition was noted in diabetic patients with more pronounced atherosclerotic vascular changes. In this subgroup of patients, even at a certain diminution of glycemia and glucosuria, no tendency was observed toward normalization of the protein blood composition, which speaks for a more pronounced impairment of the protein metabolism and more permanent and stable changes in the protein composition of the blood. In five patients in whom nadisan administration was not accompanied by a good therapeutic effect (subjective data and the sugar level in the blood and urine), some electrophoreograms showed, under dynamic investigation, an increase of dysproteinemia (hypoalbuminemia and increase of beta- and gamma-globulins).

Conclusions

1. In diabetes mellitus patients changes occur in the protein-proteid composition of the blood which are manifested as dysproteinemia, dyslipoproteidemia and dysglucoproteidemia, as the result of impairment of neuroendocrine regulation of metabolic progressions. These changes depend on the seriousness of the diabetic condition, on the presence and extent of atherosclerosis and on the therapy employed.

2. In uncomplicated diabetes there is manifested an increase of alpha$_2$-globulin, beta-lipoproteid and alpha$_2$-glucoproteid. In diabetic patients with pronounced symptoms of atherosclerosis there is manifested considerable hypoalbuminemia, increase of globulin fractions (mainly at the
expense of alpha₂-, beta and, to a lesser extent, gamma-globulins) as well as a stable increase in the content of beta-lipoproteid and alpha₂-glucoproteids.

3. The total protein content of the blood serum in patients suffering from uncomplicated diabetes and atherosclerosis was within normal limits. The level of hexoses bound with proteins and hypercholesterinemia proved to be higher in patients suffering from diabetes and atherosclerosis.

4. The attainment of a state of compensation in diabetes by means of complex therapy (diet, insulin, sulfanilamide, preparations) is accompanied frequently by approximate or in a number of cases complete normalization of the protein blood content. In 19 out of 31 patients with pronounced atherosclerotic changes, the use of sulfanilamide preparations produced no discernible tendency toward normalization of the protein composition of the blood.

5. The data on the protein-proteid composition of the blood in diabetic patients has important prognostic value and can be utilized for the control of treatments, which is particularly important in the use of the new sulfanilamide preparations. The results of study of lipoproteids and glucoproteids have definite diagnostic value in the elucidation of vascular affections (considerable and stable increase of beta-lipoproteid, alpha₂-glucoproteid, and hexoses bound with proteins).

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