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EFFECT OF PROTEIN DEFICIENCY ON THE METABOLISM OF
GLYCOPROTEINS AND GLYCOSAMINOGlyCANS IN ALBINO RAT SKIN

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1. The total content of neutral sugars in skin of the weanling albino rats kept
on the protein-deficient diet was increased by about 40%; this was mainly due to the
increased concentration of galactose. The content of sialic acid was increased by
about 20%. The collagen nitrogen was decreased significantly, with a concomitant
increase of non-collagen nitrogen. At the same time, the content of sulphated glycos-
aminglycans in skin was significantly decreased and that of non-sulphated glycos-
aminglycans was increased.

2. Protein-deficient diet enhanced the activities of the protein-bound carbo-
hydrate-degrading lysosomal hydrolases, viz. cathepsin D (EC 3.4.4.23), N-acetyl-
-β-D-glucosaminidase (EC 3.2.1.30) and β-D-glucuronidase (EC 3.2.1.31) both in
liver and skin. The activity of liver hyaluronidase (EC 3.2.1.35) was also increased
upon limitation of protein supply.

3. The changes observed in skin were accompanied by increased concentration
of the protein-bound hexoses, hexosamines and sialic acids in serum, and of hexos-
amine and uronic acid in urine. The serum fucose remained unchanged.

The effect of protein deficiency on certain constituents of glycoproteins and
glycosaminoglycans was studied in different tissues. Isotopic studies of Taoka &
Fillios (1971) and Becker (1971) indicated that protein deficiency enhanced synthesis
of hepatic glycoproteins and glycosaminoglycans. Menon et al. (1976) reported that
the total content of hexose in liver, kidney, brain and heart increased significantly
while the level of sialic acid differed in tissues of rats fed protein-deficient diet. Va-
santha (1970) observed that the content of total nitrogen and total collagen decreased
and that of hexosamine increased in the skin of rats kept on protein-deficient diet.
Consequently, the hexosamine/collagen ratio, considered as an index of ageing, was
higher on protein-deficient diet. However, it has not been conclusively established
to what extent protein deficiency affects the turnover of glycoproteins and glycos-
aminoglycans in skin. This problem is the subject of the present investigation. A part of this work was presented at the 46th Annual General Meeting of the Society of Biological Chemists of India held at Madras in September 1977.

MATERIALS AND METHODS

*Animals.* Twenty-one-day-old weanling albino rats were pair-fed for five days with a basal diet composed of: casein (20%), sucrose (30%), starch (40%), groundnut oil (6%) and salt mixture (4%) (Hubbel et al., 1937). Then the animals were divided into two groups of 36 each. The first group was allowed to continue with the basal control diet while the second group was pair-fed with a protein-deficient (6%) diet containing 54% starch. Water was supplied ad libitum and both groups were given the required vitamins. Food consumption was recorded daily and body weight was taken weekly. At the end of the experimental period (21 days), 24 hour urine was collected, the rats were killed by decapitation, the blood was collected, and livers and skins were immediately removed. The skins were unhaired with a razor, fleshed, washed and weighed (Table 1). For determination of enzymatic activities, tissues from six animals from either group were taken and used as individual samples. For other determinations, tissues from five animals were pooled to make one sample, thus making six combined samples in either group.

*Analysis of serum and urine.* The serum protein-bound hexose, hexosamine, sialic acid and fucose were determined as described by Winzler (1968). Urinary total hexosamine was measured by the method of Elson & Morgan (1933) as modified by Rimington (1940). Urinary uronic acid was assayed according to Ritchie et al. (1977), and creatinine using the Jaffe reaction (Picou et al., 1965).

*The assay of enzymatic activities in liver and skin.* Liver was homogenized in ice-cold 0.2 M-sucrose (1:10, v/v) in a glass-teflon homogenizer at 1500 rev./min for 30 s and in a part of the homogenate hyaluronidase was determined using hyaluronic acid (sodium salt) as a substrate in 0.1 M-acetate buffer, pH 3.8 (Kawai & Anno, 1971) and estimating N-acetyl hexosamine liberated by the method of Reissig et al. (1955). The rest of the homogenate was diluted 5-fold with ice-cold distilled water. After further homogenization at 1500 rev./min for 30 s aliquots of this preparation were used for determination of N-acetyl-β-D-glucosaminidase (Rosenblit et al., 1974), β-D-glucuronidase (Delvin & Gianetto, 1970), and cathepsin D (Sapolsky et al., 1973).

Skin was homogenized as described by Steigerwald & Bartholomew (1973): it was cut into pieces (about 5 microns in diameter) in a freezing microtome, homogenized in 0.15 M-potassium chloride, centrifuged for 20 min at 14–500 g in a refrigerated centrifuge and in the supernatant the activities of N-acetyl-β-D-glucosaminidase, β-D-glucuronidase (Steigerwald & Bartholomew, 1973) and cathepsin D (Sapolsky et al., 1973) were determined.

*Protein* was estimated by the method of Lowry et al. (1951).

*Estimation of glycoproteins and glycosaminoglycans in skin.* The skin was defatted by extraction at 60°C with methanol/ether (3:1, v/v) and chloroform/methanol.
(1:1, v/v), twice with each solvent for 2 h. The defatted samples were dried to constant weight. For estimation of carbohydrate components of glycoproteins, the method of Wagh et al. (1973) was used, except that ethanol was applied to deproteinize the digest instead of trichloroacetic acid. After papain digestion of dry defatted tissue and deproteinization of the digest by ethanol, the solution was evaporated in vacuum. For estimation of total neutral sugars and hexosamines, the sample was hydrolysed in 0.1 m-HCl in the presence of polysulphonated resin (Dowex 50W-X8, 200-400 mesh) in a sealed glass tube at 100°C for 20 h. The contents, i.e. the hydrolysate and the resin, were quantitatively introduced into an empty column (10 mm in diameter), then neutral sugars were eluted with deionized water and hexosamines with 2 m-HCl (Chandrarajan & Bose, 1965). The content of neutral sugars was estimated by the phenol-sulphuric acid method (Dubois et al., 1956), and hexosamines by the modified method of Rimington (1940). Monosaccharides were separated by the ion-exchange column chromatography (Walborg et al., 1965), and the fractions obtained were analysed for sugar content using the aniline/acetic acid/orthophosphoric acid method as described by Walborg & Christensson (1965). Sialic acid was determined by the thioarbituric acid method of Warren (1959) after hydrolysis of the sample with 0.05 m-H2SO4 at 80°C for 1 h.

Total nitrogen was estimated by the Kjeldhal micro method. The collagen and non-collagen nitrogen were determined as described by Anastassiadis et al. (1972).

Dry defatted skins were analysed for non-sulphated, monosulphated and highly sulphated glycosaminoglycans according to Mier & Wood (1969). The results were statistically evaluated using Student's t test.

RESULTS AND DISCUSSION

In weanling albino rats kept on a protein-deficient diet, total body weight and skin weight were appreciably decreased (Table 1) as compared with the control animals.

Table 1

Influence of protein deficiency on total body and skin weight of albino rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight (g)</th>
<th>Final skin weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial</td>
<td>final</td>
</tr>
<tr>
<td>Control</td>
<td>29.2±1.4</td>
<td>67.3±3.8</td>
</tr>
<tr>
<td>Protein-deficient</td>
<td>29.0±1.3</td>
<td>43.2±3.7</td>
</tr>
</tbody>
</table>

As can be seen from Table 2, the content of total neutral sugars in skin of rats receiving protein-deficient diet was increased by 42%. The hexosamine content was but slightly increased. The glycoprotein index was elevated due to sialic acid, present
in the majority of glycoproteins. The content of this compound in the animals on protein-deficient diet was increased by 18.6%. The significant increase in the content of total neutral sugars was due mainly to the increased concentration of galactose while the content of xylose was even decreased. The significant reduction of the protein content in the skin was due to the lowered collagen content; at the same time, non-collagen nitrogen was increased.

Table 2

Influence of dietary protein deficiency on the content of carbohydrate constituents and nitrogen in the skin of albino rats

Composition of the diet and sampling as described in Methods. Total neutral sugars are expressed as galactose, hexosamine as galactosamine, sialic acid as N-acetylmuramic acid, and glycosaminoglycans as glucuronic acid, all per 100 mg of dry defatted skin. Values are means ±S.E. of six samples, with five skins in each sample.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Diet</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>protein-deficient</td>
<td></td>
</tr>
<tr>
<td>Total neutral sugars (μg)</td>
<td>1328 ±58.3</td>
<td>1897 ±90.7*</td>
<td></td>
</tr>
<tr>
<td>Galactose (μg)</td>
<td>616.3 ±28.4</td>
<td>1151 ±38.5*</td>
<td></td>
</tr>
<tr>
<td>Mannose (μg)</td>
<td>438.1 ±19.1</td>
<td>509 ±18.4*</td>
<td></td>
</tr>
<tr>
<td>Glucose (μg)</td>
<td>205.4 ± 6.4</td>
<td>226 ± 6.9*</td>
<td></td>
</tr>
<tr>
<td>Xylose (μg)</td>
<td>68.5 ± 2.9</td>
<td>22.5 ± 1.2*</td>
<td></td>
</tr>
<tr>
<td>Hexosamine (μg)</td>
<td>416 ±14.7</td>
<td>428 ±12.5</td>
<td></td>
</tr>
<tr>
<td>Sialic acid (μg)</td>
<td>68 ± 2.5</td>
<td>81 ± 4.5*</td>
<td></td>
</tr>
<tr>
<td>Total glycosaminoglycans (μg)</td>
<td>154.5 ±2.41</td>
<td>166.5 ±5.37*</td>
<td></td>
</tr>
<tr>
<td>— non-sulphated (μg)</td>
<td>64.1 ±2.24</td>
<td>92.6 ±2.16*</td>
<td></td>
</tr>
<tr>
<td>— mono-sulphated (μg)</td>
<td>65.4 ±1.78</td>
<td>55.4 ±3.81*</td>
<td></td>
</tr>
<tr>
<td>— highly sulphated (μg)</td>
<td>25.0 ±0.41</td>
<td>18.5 ±0.93*</td>
<td></td>
</tr>
<tr>
<td>Total nitrogen (mg)</td>
<td>15.6 ±0.21</td>
<td>14.4 ±0.18*</td>
<td></td>
</tr>
<tr>
<td>Collagen nitrogen (mg)</td>
<td>12.4 ±0.26</td>
<td>9.7 ±0.15*</td>
<td></td>
</tr>
<tr>
<td>Non-collagen nitrogen (mg)</td>
<td>3.2 ±0.16</td>
<td>4.7 ±0.27*</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.01.

Data included in Table 2 show that limitation of protein in the diet resulted in the increase in non-sulphated glycosaminoglycans whereas the content of mono- sulphated and highly sulphated glycosaminoglycans was significantly decreased.

The activities of N-acetyl-β-D-glucosaminidase, β-D-glucuronidase and cathepsin D were increased in liver and skin of the “protein deficient” group (Table 3). In liver, the activity of hyaluronidase was also significantly enhanced.

The levels of protein-bound hecose, hexosamine and sialic acid in serum were increased in the animals receiving the protein-deficient diet (Table 4), whereas the content of fucose was not altered significantly, and the urinary excretion of total hexosamines and uronic acid were significantly increased (Table 5).
Table 3

The activities of lysosomal hydrolases in liver and skin of rats kept for 3 weeks on protein-deficient diet

The results are expressed as μmoles of the product formed/h per g of protein; they are mean values from 6 animals ± S.E. For details see Methods.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Liver</th>
<th></th>
<th></th>
<th>Skin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control diet</td>
<td>Protein-</td>
<td>Control</td>
<td></td>
<td>Protein-</td>
</tr>
<tr>
<td></td>
<td>1.49</td>
<td>deficient</td>
<td>diet</td>
<td>2.04*</td>
<td>deficient</td>
</tr>
<tr>
<td></td>
<td>±0.08</td>
<td>±0.10</td>
<td></td>
<td>719.6</td>
<td>±36.0</td>
</tr>
<tr>
<td>N-Acetyl-β-d-glucosaminidase</td>
<td>1062</td>
<td>1386*</td>
<td>±43.9</td>
<td>±75.5</td>
<td>±6.40</td>
</tr>
<tr>
<td></td>
<td>±43.9</td>
<td>±75.5</td>
<td>±145.0</td>
<td>180.5*</td>
<td>±7.91</td>
</tr>
<tr>
<td>β-d-Glucuronidase</td>
<td>±6.04</td>
<td>±7.91</td>
<td>±145.0</td>
<td>180.5*</td>
<td>±7.91</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>288.1</td>
<td>362.7*</td>
<td>±288.1</td>
<td>362.7*</td>
<td>±19.9</td>
</tr>
<tr>
<td></td>
<td>±11.9</td>
<td>±19.9</td>
<td>±288.1</td>
<td>362.7*</td>
<td>±19.9</td>
</tr>
</tbody>
</table>

* P < 0.01.

The increased excretion of hexosamines and uronic acid, and the increased activities of lysosomal hydrolases suggested increased degradation of the ground substance of the skin in the animals kept on protein-deficient diet. Adhikari et al. (1971) suggested that the changes in the activities of the enzymes were due to the increased fragility of lysosomes in protein deficiency. On the other hand, the increased level of glycoproteins and non-sulphated glycosaminoglycans indicates increased synthesis of these compounds in protein deficiency. Our results corroborate the findings of Taoka & Fillios (1971) who found increased synthesis of hepatic glycoproteins during protein depletion. The decreased amount of sulphated glycosaminoglycans may thus be due to increased catabolism.

Table 4

The content of protein-bound carbohydrates in sera of rats kept on control and protein-deficient diets

For details of analytical methods and sampling see Methods. The results are expressed as mg/100 ml of serum; they are mean values ± S.E. of six samples, each containing sera of five animals.

<table>
<thead>
<tr>
<th>Sugars</th>
<th>Control</th>
<th>Protein-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose</td>
<td>147.55 ± 3.98</td>
<td>166.28 ± 3.88*</td>
</tr>
<tr>
<td>Hexosamine</td>
<td>95.09 ± 4.10</td>
<td>125.14 ± 4.26*</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>96.13 ± 3.53</td>
<td>109.38 ± 2.38*</td>
</tr>
<tr>
<td>Fucose</td>
<td>8.34 ± 0.41</td>
<td>8.81 ± 0.47</td>
</tr>
</tbody>
</table>

* P < 0.01.
Table 5

Urinary excretion of hexosamine and uronic acid by rats kept on control and protein-deficient diet

The results are expressed as mg/100 mg of creatinine; they are mean values ± S.E. of six samples, each containing urine from five animals. For details of sampling see Methods.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control diet</th>
<th>Protein-deficient diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexosamine</td>
<td>80.45 ± 3.75</td>
<td>101.65 ± 4.15*</td>
</tr>
<tr>
<td>Uronic acid</td>
<td>9.68 ± 0.41</td>
<td>12.64 ± 0.46*</td>
</tr>
</tbody>
</table>

* P < 0.01.

In the skin of protein-deficient rats the turnover of glycoproteins and glycosaminoglycans seems to be altered. It is likely that, in protein deficiency, the synthetic mechanism of glycoproteins and non-sulphated glycosaminoglycans may predominate over the enhanced catabolism.

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REFERENCES


WPLYW NIEDOBoru BIaŁKA NA METABOLIZM GLIKOPROTEIDÓW
I GLIKOZAMINOGLIkiANÓW W SKÓRZE SZCZUROw ALBINÓSÓw

Streszczenie

1. W skórze szczurów albinów karminowych od 21 dnia życia dieć ubogą w białko, całkowita zawartość cukrów obojętnych była zwiększona o ok. 40%, przy czym wzrost ten był spowodowany głównie zwiększeniem stężenia galaktose. Zawartość kwasu siłowego była zwiększona o ok. 20%. Azot kolagenowy był znacząco zmniejszony, z równoczesnym wzrostem azotu niekolagenowego. Jednocześnie zawartość w skórze zawierających siarkę glikozaminoglikanów była zmiennie zmniejszona, a zawartość glikozaminoglikanów nie zawierających siarki — zwiększona.
2. Zarówno w wątrobie, jak i w skórze szczurów karminowych dieć ubogą w białko zwiększała się aktywność hydrołazu lizozomalnych, rozkładających związane z białkiem cukry, a mianowicie: katapsyny D (EC 3.4.4.23), N-acetylo-β-D-glukozaminidazy (EC 3.2.1.30) i β-D-glukuronidazy (EC 3.2.1.21). Ograniczenie białka w diecie powodowało również zwiększenie aktywności hialuronidazy (EC 3.2.1.35) w wątrobie.
3. Zmianom obserwowanym w skórze towarzyszyło zwiększenie stężenia w surowicy związanych z białkiem heksos, heksosamin i kwasów siłowych, oraz stężenia w moczu heksosamin i kwasu uronowego. Zawartość fukozy w surowicy nie była zmieniona.

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