Factors Influencing Serum Glycoprotein Levels in the Mouse and Rat

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SUMMARY

The changes in serum protein-bound hexose concentration following the injection of papain, hyaluronidase, estriol, ethylene and propylene glycol, Aminopterin, urethan, turpentine, adrenocorticotropic hormone, and homogenates of normal and autolyzed liver have been examined. Neither stimulation nor suppression of cell proliferation had any marked effect on serum glycoproteins. Both normal and autolyzed liver were equally effective in producing a rise in serum glycoprotein levels. A rise occurred after sham operation but not after partial hepatectomy.

INTRODUCTION

It is well known that the serum glycoprotein content rises in a wide variety of infectious and neoplastic diseases (23, 34). Neither the cause nor the significance of this rise is known, and it is still uncertain whether it is related to tissue necrosis (9, 13, 14) or to cell proliferation (4, 20, 30). However, glycoproteins have been claimed to be depolymerization products of the ground substance (7), and this still remains a possible source. It may be that more than one of these theories is correct, and it was intended in the present work to examine the various possibilities.

MATERIALS AND METHODS

Inbred albino mice and Wistar rats were employed in this study. Male animals were used throughout, except for the groups given estriol and their controls. Some increase of serum glycoproteins with age has been reported in man (24, 35). Only mature mice, 20-25 gm, were used. The animals were bled 48 hr after the start of an experiment, unless stated otherwise. Serum was obtained from 26 normal mice during the course of the experiments. The following dose schedules were followed for the various groups: 1 ml of sterile Ringer's saline i.p.; 2 s.c. doses of 0.1 ml of fresh oil of turpentine; 15 IU (20 µg) of hyaluronidase s.c. (bled 24 hours later); a saline suspension of papain (2 mg) s.c.; a single s.c. dose of 0.125 µg of estriol in 0.25 ml of propylene glycol into adult female mice, oophorectomized by the dorsal route 2 weeks previously (bled 24 hours later); an s.c. dose of 0.125 µg of estriol in 0.25 ml of arachis oil into adult oophorectomized mice; a single s.c. dose of ethylene glycol, 0.25 ml of a 1:5 dilution in saline (bled 24 hours later); an s.c. dose of 75 µg of Aminopterin (4-aminopteroyl glutamic acid) in 0.2 ml of saline at pH 8; an s.c. dose of 20 mg of urethan, twice daily for 2 days; two s.c. doses of adrenocorticotropic hormone (each 25 ml'), the second dose 24 hr after the first; and liver homogenates (the liver was removed from a normal mouse and homogenized with an equal volume of saline containing 0.5% ampicillin. The autolyzed liver was prepared by ligating 2 liver lobes as for partial hepatectomy, removing them 24 hr later and homogenizing. To suppress bacteria, 100 mg of ampicillin were given before and after operation).

Partial hepatectomy was performed on rats, removing two-thirds of the liver. Sham operations were also performed in which the abdomen was opened and the liver lobes were manipulated and replaced, together with a small piece of liver to simulate the stump of dead tissue left after partial hepatectomy. Ten rats were used in each group, and estimations of sialic acid, fucose, protein-bound hexose, and total protein content were performed on the serum from each rat.

Protein was determined by the biuret method, using a single batch of Versatol as a standard. Protein-bound hexose was estimated by the orcinol reaction (35), using a standard containing equal proportions of galactose and mannose. Fucose was estimated by the method of Dicke and Shettles (35), using L-fucose as the standard. Sialic acid was estimated by the method of Svennerholm (32) as modified by Rice (26), using N-acetyl neuraminic acid as the standard. This method is stated to be satisfactory for serum (32).

RESULTS

The estimation of protein-bound hexose was used as an index of glycoprotein levels in the experiments with mice, and the results are given in Table 1. Animals injected with sterile saline did not show any difference in serum glycoprotein levels from normal controls. Neither papain nor hyaluronidase, when allowed to act for 24 hr, had any significant effect, but ulceration of the skin was beginning to occur in mice given papain 48 hours previously, and the rise in this group was significant (P < 0.01 against saline controls). The effect of protein catabolism induced by a 24-hour fast was tried; food was withheld, but water was given ad libitum. No significant alteration occurred in glycoprotein levels.

To test the effect of tissue proliferation without necrosis, rapid growth was induced in the uterus and vagina by injecting estrogen after oophorectomy. The estriol solution, in both normal and spayed mice, and also the vehicle used (propylene glycol), caused considerable falls in the hexose levels. The fall produced by the vehicle is almost certainly due to its hepatic toxicity (6); ethylene glycol had a similar effect, and both glycols caused marked vacuolation of the parenchymal cells. The mean serum hexose content for the group of normal females treated with estriol differs significantly from spayed mice receiving estriol (P < 0.005) and from propylene glycol controls (P < 0.001).
Factors Influencing Serum Glycoproteins

The Effect of Various Experimental Procedures on the Serum Protein-bound Hexose Concentration in Mice

<table>
<thead>
<tr>
<th>Experimental procedure</th>
<th>No. of mice</th>
<th>Total serum protein (gm %) ± S.E.</th>
<th>Protein-bound hexose (mg %) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>26</td>
<td>5.18 ± 0.10</td>
<td>137 ± 6</td>
</tr>
<tr>
<td>Saline controls</td>
<td>10</td>
<td>4.94 ± 0.24</td>
<td>136 ± 4</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>11</td>
<td>4.95 ± 0.10</td>
<td>125 ± 7</td>
</tr>
<tr>
<td>Papain (24 hours)</td>
<td>9</td>
<td>5.34 ± 0.29</td>
<td>142 ± 5</td>
</tr>
<tr>
<td>Papain (48 hours)</td>
<td>10</td>
<td>5.52 ± 0.14</td>
<td>168 ± 7</td>
</tr>
<tr>
<td>Fasting</td>
<td>12</td>
<td>5.67 ± 0.16</td>
<td>140 ± 6</td>
</tr>
<tr>
<td>Estriol + propylene glycol</td>
<td>15</td>
<td>5.20 ± 0.20</td>
<td>114 ± 4</td>
</tr>
<tr>
<td>Estriol + glycol</td>
<td>14</td>
<td>4.90 ± 0.14</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>15</td>
<td>4.90 ± 0.01</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>9</td>
<td>5.36 ± 0.15</td>
<td>109 ± 6</td>
</tr>
<tr>
<td>Estriol + arachis oil in spayed females</td>
<td>9</td>
<td>6.15 ± 0.13</td>
<td>144 ± 13</td>
</tr>
<tr>
<td>Arachis oil in spayed females</td>
<td>9</td>
<td>6.20 ± 0.24</td>
<td>152 ± 6</td>
</tr>
<tr>
<td>Turpentine</td>
<td>16</td>
<td>5.50 ± 0.20</td>
<td>183 ± 6</td>
</tr>
<tr>
<td>Turpentine + aminopterine</td>
<td>11</td>
<td>5.44 ± 0.22</td>
<td>198 ± 9</td>
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<tr>
<td>Aminopterine</td>
<td>12</td>
<td>5.71 ± 0.14</td>
<td>127 ± 6</td>
</tr>
<tr>
<td>Turpentine + urethane</td>
<td>11</td>
<td>5.30 ± 0.13</td>
<td>165 ± 5</td>
</tr>
<tr>
<td>Urethane</td>
<td>12</td>
<td>5.36 ± 0.14</td>
<td>125 ± 4</td>
</tr>
<tr>
<td>Turpentine + ACTH²</td>
<td>14</td>
<td>5.30 ± 0.15</td>
<td>178 ± 5</td>
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<tr>
<td>Normal liver (30 mg)b</td>
<td>16</td>
<td>5.49 ± 0.17</td>
<td>145 ± 6</td>
</tr>
<tr>
<td>Normal liver (100 mg)b</td>
<td>10</td>
<td>5.23 ± 0.21</td>
<td>162 ± 7</td>
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<tr>
<td>Autolyzed liver (20 mg)b</td>
<td>16</td>
<td>5.80 ± 0.21</td>
<td>161 ± 9</td>
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<tr>
<td>Autolyzed liver (100 mg)b</td>
<td>10</td>
<td>5.57 ± 0.19</td>
<td>159 ± 6</td>
</tr>
</tbody>
</table>

² Adrenocorticotropin.
b Dry weight.

As it is possible that a liver affected by propylene glycol would not be able to respond to a stimulus such as cell proliferation, a second experiment was performed using arachis oil as solvent for the estriol. There was no significant difference between the two groups (P > 0.10) or from control values. In both experiments sections of the vagina from mice given estriol showed many dividing cells.

Turpentine abscesses will raise serum glycoprotein levels, and therefore a group of mice was injected with aminopterine and turpentine, to suppress any associated proliferative response. This antimitabolite had no effect on the rise in protein-bound hexose levels, the values for groups given turpentine alone, and turpentine with Aminopterin, being significantly different from control values (P < 0.001) but not from each other. The experiment was repeated with urethan and turpentine; in this instance the protein-bound hexose values also showed a significant difference from control levels (P < 0.01) and from urethan (P < 0.001). The slight fall occurring in glycoprotein levels in those mice receiving either urethan or Aminopterin only is again probably due to mild liver damage, as sections showed vacuolation of parenchymal cells. In sections of the turpentine abscesses, both from mice receiving Aminopterin and from those receiving urethan, no evidence of any proliferative response was seen, although polymorphonuclear leukocytes were present.

A group of mice received turpentine and ACTH (adrenocorticoterpin), in order to depress the inflammatory response and test the effect of endogenous corticosteroids. No significant alteration occurred in the glycoprotein level.

To gain some idea of the possible role of tissue necrosis, liver homogenates were injected. A small dose of normal liver had no effect on the serum protein-bound hexose, although a similar dose of autolyzed liver produced a just significant effect (P < 0.05). With a much larger dose both normal and autolyzed liver produced a similar and significant difference from control values (P < 0.025 and P < 0.05, respectively).

The effect of sham operation and partial hepatectomy was tested in rats (Table 2), and it can be seen that sham operation induced a statistically significant rise in serum glycoprotein levels (P < 0.001 for hexose), whereas partial hepatectomy had no effect, and that there was no significant difference in the response of serum fucose, sialic acid, or protein-bound hexose levels to those procedures.

DISCUSSION

Several theories have been proposed to account for the rise in serum protein-bound carbohydrate fractions occurring in infection, cancer, and nonspecific stress:

1. Catchpole and his colleagues considered both tissue and serum glycoproteins to be depolymerization products of the ground substance (7, 12, 25). If this theory were correct then the injection of proteolytic enzymes would produce a rapid rise in serum levels.

Robert et al. (27) tried the effect of a number of enzymes, given i.v. on the serum haptoglobin concentration of rabbits. They obtained a marked rise at 24 hr with papain and a moderate rise with hyaluronidase, greatest at about 10 hr, but obtained no effect with trypsin. Others (28) gave trypsin s.c. and found the plasma hexosamine level beginning to rise at 24 hr. In the present work no change occurred in serum protein-bound hexose 24 hr after the injection of papain and hyaluronidase.

It had also been claimed that a rise occurred in haptoglobin in animals on a restricted diet (27, 33), which might be accounted for by the catabolism of tissue protein. In fact, no change was observed in the serum glycoproteins of mice deprived of food for 24 hr, while others (2) found a marked fall in plasma hexosamine levels in fasting rats, even after injection with turpentine.

Although small amounts of glycoproteins can be synthesized by extrahepatic tissues (8, 18), it is evident that most, if not all, of the serum glycoproteins are produced by the liver (17, 19, 21). The results given in Table 2, which indicate a failure of the normal glycoprotein response to trauma, are in general agreement with the observations of others (10, 18, 21) and would tend to confirm that the liver is the site of synthesis. Glycoproteins constitute some 10% of the serum proteins and have a half life of about 2 days (3); there is no evidence to indicate that extrahepatic tissues are the source of the relatively large amounts appearing after trauma. The fact that proteolytic enzymes and fasting do not stimulate a rise in serum glycoprotein levels would support this view.

Although there is some evidence that the different serum glycoproteins do not alter in parallel (13, 29, 36), there appears to be no marked quantitative difference in the ratio of hexose, fucose, and sialic acid in rats following an increase due to trauma (Table 2).
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2. It has been considered by some workers that the rise in serum glycoprotein levels is a result of tissue proliferation whether this be tumor tissue, or the normal repair processes (4, 5, 20, 30). However, tissue growth and necrosis are almost invariably associated, for repair occurs even in the presence of tissue destruction and tumors invade normal tissue and contain areas of necrosis.

This hypothesis was therefore investigated in 2 ways: (a) by the stimulation of cell proliferation without associated tissue necrosis, using estrogens. It is evident from Table 1 that such growth had no effect on serum glycoprotein levels; this is clearly seen in those experiments where arachis oil was used, as neither estriol nor its vehicle produced any significant effect. (b) The second way was by the suppression of cell proliferation using antimitabolites and ACTH. Neither Aminopterin, urethan, nor ACTH had any significant effect in reducing the response of serum glycoproteins to subcutaneous injections of turpentine. Derache and Mariel (11) claimed that there was a rise in hexose levels 24 hours after partial hepatectomy, and reported that this was inhibited by cortisone. Darcy (10) reported that cortisone and cortisol, in large doses, caused slight inhibition of the rise in serum concentration of an \( \alpha \)-glycoprotein produced by turpentine, but Heppleston and Keyser found that cortisone did not affect the rise due to silica granuloma (14), and ACTH was not found to have any effect on the elevation of mucoproteins following epidermal damage (16).

Seltlar et al. (31) claimed that there was a significant rise in serum glycoprotein levels in the last trimester of pregnancy and adduced this as evidence that cell proliferation was responsible for the rise. However, placental infants are not uncommon in late pregnancy, and such tissue destruction could account for at least part of the rise; further, fetal growth is most rapid in the first half of pregnancy. Others (15, 22) found no rise in normal pregnancy, but only in toxemia of pregnancy (22), in which small infants are common.

It would seem unlikely that cell proliferation or the inflammatory response have any significant effect on serum glycoprotein levels; otherwise, estrogens, antimitabolites, and steroids would be expected to have some influence.

3. Other workers have put forward evidence to suggest that it is tissue destruction alone, rather than growth, which affects serum glycoprotein levels (1, 9, 13). Darcy (9) found that dead kidney or liver tissue left in situ provoked a considerable rise in an \( \alpha \)-glycoprotein; he did not think that infection itself caused this rise, as rats with a Walker carcinaoma treated with tetracycline also showed the rise. Recently Apsey et al. (1) also found a rise with injections of normal liver and tumor extracts, and this was more marked when the extracts were administered with adjuvant. The effect in mice of injecting liver homogenates (Table 1) suggests that normal tissue components are capable of stimulating a rise in the serum glycoproteins. It is possible that such a homogenate is not directly responsible for the effect on the glycoproteins, but might produce some reaction in the recipients which in turn affects the serum levels. However, this would still indicate that normal, as well as autolyzed, liver contains factors which can cause a rise in serum glycoprotein levels, whether directly or indirectly. It is possible that this effect could be produced by stimulation of hepatic synthesis, as it seems likely, on the evidence at present available, that the elevation in serum glycoprotein levels which follows trauma, cancer, and infections is due to their increased synthesis rather than to a reduced rate of utilization or destruction (36).

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REFERENCES

8. Coffey, J. W., Miller, O. N., and Sellinger, O. Z. The Metab-

#### Table 2

<table>
<thead>
<tr>
<th>Serum component</th>
<th>Normal</th>
<th>After partial hepatectomy</th>
<th>After sham operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein-bound hexose (mg %)</td>
<td>134 ± 8</td>
<td>130 ± 6</td>
<td>128 ± 4</td>
</tr>
<tr>
<td>Fuose (mg %)</td>
<td>17.6 ± 0.6</td>
<td>16.2 ± 0.3</td>
<td>17.2 ± 0.5</td>
</tr>
<tr>
<td>Sialic acid (mg %)</td>
<td>70.4 ± 3.0</td>
<td>67.4 ± 2.3</td>
<td>67.5 ± 1.4</td>
</tr>
<tr>
<td>Protein (gm %)</td>
<td>5.47 ± 0.15</td>
<td>5.24 ± 0.11</td>
<td>5.39 ± 0.12</td>
</tr>
</tbody>
</table>
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