Hematopoietic and Nitrogen-Sparing Effect of Preparations from Hog Gastric Mucosa with High Content of Blood Group Substances

By ANGELO CRESSERI, AURELIO CANTONE, FRANCO PICCININI AND VITTORIO CAPRARO

IN THE COURSE of investigations to find a laboratory test for intrinsic factor activity, it has been observed that a mucin preparation showed an evident hematopoietic effect in mice and rats made anemic by intraperitoneal injection of phenylhydrazine hydrochloride. The mucin preparation was obtained from hog gastric mucosa by trichloroacetic acid deproteinization of the aqueous extract followed by acetone precipitation. It was active as intrinsic factor in humans. Further investigations, however, have shown that the hematopoietic effect was not due to an increase of vitamin B₁₂ or Fe intestinal absorption. Therefore, it could not be related to the intrinsic factor content of the preparation. This conclusion agrees with the observations of Rosenblum et al. and Chow et al. on the inactivity of hog intrinsic factor in the rat, a species that probably needs a specific intrinsic factor.

It may be supposed that the above referred antianemic effect is exerted through a sparing action on nitrogen metabolism. Therefore, in order to identify the component (or components) responsible for the reported activities, experiments were undertaken to test for hematopoietic and nitrogen-sparing action a fraction, with high content of A and H blood group substances obtained from the mucin preparation previously used.

MATERIALS AND METHODS

Preparation of mucin fractions with high content of A and H blood group substances. The hog gastric mucin was suspended in 90 per cent phenol in water (w/v), and the insoluble residue discarded by centrifugation. To the supernatant, stirred mechanically, a mixture of equal parts of absolute ethanol and 90 per cent phenol was added slowly dropwise until the final ethanol concentration reached 10 per cent by volume. The precipitate was collected by centrifugation and dried with ethanol and ether. At the electrophoretic analysis (Perkin Elmer Co. apparatus, mod. 38, 2 ml. cell) the fraction shows the prevailing presence (fig. 1) of a component with a mobility (descending) of about -1.6 x 10⁻⁶ cm.² sec⁻¹ V⁻¹. The value is in close agreement with the mobility reported in the literature for similar A and H blood group substance preparations from hog gastric mucosa.

This fraction inhibits the agglutination of group A red blood cells by human anti-A serum (titer 1:256 diluted 1:64) at dilutions of 1:10⁻⁷ - 1:7 x 10⁻⁸ and the agglutination of group O red blood cells by eel anti-H serum (titer 1:256 diluted 1:64) at dilutions of...
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Fig. 1.—Electrophoretic pattern (ascending) after 120 min. of the mucin fraction with high content of A and H blood group substances used in the present experiment (1% (w/v) soln., at 6 V/cm., phosphate pH 6, I = 0.2).

The mucin fraction shows a slight binding power on vitamin B₁₂ (0.08-0.16 μg./mg.) according to the determination of the "semimaximum inhibition dose" by cup plate method with E. coli 113-3. The substance presents a good Bifidus factor activity (about 1.66 units/mg.).

Antianemic test. Male albino rats weighing 100 to 120 Gm., fed during all the course of the experiment a basal diet deficient in vitamin B₁₂, were used. On the seventh day after the beginning of the diet the animals were injected intraperitoneally with phenylhydrazine hydrochloride (5 mg./100 Gm. body weight). On the fifth day after the injection, when red blood cell counts dropped to about 50 per cent of the initial values, the rats were divided in three groups. Group A (control group) received the basal diet only; group B, the basal diet supplemented with vitamin B₁₂, 1 μg. per animal per day by gastric tube; group C, the basal diet supplemented with the same dose of vitamin B₁₂ and 7 mg. of the mucin fraction with high content of A and H blood group substances per animal per day by gastric tube. A larger number of animals in groups B and C, in comparison with control group A, has been used in order to ascertain also small differences between them in the hematologic response. Red blood cell counts were performed on the second and sixth day after the beginning of the different treatments. The antianemic effect is evaluated from the increase in red blood cell counts on the sixth day of treatment. The increase is calculated as the difference between the values of red blood cells in millions/cu. mm. on the sixth day after the beginning of the treatment and the corresponding values on the fifth day after phenylhydrazine hydrochloride injection.

Nitrogen sparing action test. Male albino rats, about 100 Gm. body weight, fed during all the course of the experiment a diet deficient in vitamin B₁₂, were used. On the seventh day after the beginning of the diet the animals were divided in three groups. Group A (control group) received the basal diet only; group B, the basal diet and cortisone* by

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* Range of different batches of the fraction tested.
† "Cortone acetate," Merck & Co.


Table 1.—Antianemic Effect of the Mucin Fraction with High Content of A and H Blood Group Substances Obtained from Hog Gastric Mucosa

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>R.B.C. increase (millions/cu.mm.)</th>
<th>Increase in R.B.C. (millions/cu.mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Controls: basal diet</td>
<td>20</td>
<td>3.01 ± 0.89</td>
<td>1.91 ± 1.47</td>
</tr>
<tr>
<td>B) Basal diet, Vitamin B,12</td>
<td>97</td>
<td>3.04 ± 0.98</td>
<td>2.38 ± 1.38</td>
</tr>
<tr>
<td>C) Basal diet, Vitamin B,12 and mucin fraction</td>
<td>92</td>
<td>2.82 ± 0.96</td>
<td>3.66 ± 1.34</td>
</tr>
</tbody>
</table>

R.B.C. increase in group B vs. group C: t* = 6.36
D.f. † = 187
P < 0.001

* t = Student 't' test.
† D.f. = number of degrees of freedom.

daily intraperitoneal injection at a dose of 20 mg. Kg.; group C, the basal diet supplemented with 0.5 per cent of the mucin fraction with high content of A and H blood group substances, and cortisone by intraperitoneal daily injection at the same dose, as in group B.

Previous experiments had shown that nitrogen excretion in control and cortisone-treated rats begins to differ only on the sixth or seventh day of treatment. Therefore, on the seventh day the animals were housed in individual metabolism cages. Twenty-four-hour diet consumption was noticed and complete 24-hour urine and feces collection performed on the seventh, eighth, ninth, tenth and eleventh days. Nitrogen determinations on diet, urine, and feces samples were performed according to Kjeldhal as modified by King. The nitrogen utilization coefficient was calculated from the ratio

\[
\text{Nitrogen Utilization Coefficient} = \frac{N_1 - N_e}{N_1}
\]

where \(N_1\) = nitrogen intake with the diet (mg./100 Gm. body weight) and \(N_e\) = total excreted nitrogen (mg./100 Gm. body weight).

RESULTS AND DISCUSSION

Table 1 shows the results of the antianemic test. No significant differences exist between the red blood cell counts on the fifth day after phenylhydrazine hydrochloride injection. Therefore, the three experimental groups may be considered affected by the same degree of anemia. On the contrary, significant differences are observed in the red blood cell increases on the sixth day after the beginning of the treatment, especially between the group treated with vitamin B,12 and the group treated with vitamin B,12 plus the mucin fraction.

These findings are consistent with the hypothesis already referred to, that in mucin preparations from hog gastric mucosa one or more substances are present that are able to promote the hematologic response in the anemic rat, independently of a better absorption of Fe or vitamin B,12.4
The sharp biological effect observed in the present experiment has been obtained with doses 1/3 lower than those previously used with the raw mucin preparation but administering a fraction with an A and H blood group substance content three times greater. We can, therefore, draw the conclusion that presumably the hematopoietic effect may be related to the A and H blood group substances.

The results of the nitrogen-sparing action test are reported in table 2 which shows for the different groups the mean values of N intake and of the N utilization coefficient, both for the single days and for the whole period since the seventh up to the eleventh day of experiment. In cortisone-treated rats in comparison with control animals a slightly lower nitrogen utilization, statistically not significant, is observed. On the contrary, highly significant statistically is the difference between the means of the nitrogen utilization coefficients (calculated on the whole period) of the group treated only with cortisone and the group treated with cortisone plus the mucin fraction with high content of A and H blood group substances ($t = 6.34$; D.f. = 87; $P < 0.001$). Also significant is the difference between the means of this group and the control group ($t = 5.23$; D.f. = 89; $P < 0.001$). It may be pointed out that nitrogen balance is positive in all groups, but nitrogen utilization coefficient is especially high in the group fed the mucin fraction, in spite of cortisone administration. It is not unlikely to suppose that the two biological effects shown by the mucin fraction used by us (the nitrogen-sparing effect and the antianemic effect) are two different consequences of the same mechanism. The nitrogen-sparing effect may be considered the manifestation of a general favorable action on protein synthesis. The above favorable action may be the cause of improvement in red blood cell regeneration. On the same lines we may consider the growth-promoting effect observed by other AA.15, 16 in rats treated with mucin preparations from hog gastric mucosa.

With regard to the mechanism of the favorable action on protein synthesis (as shown by the hematopoietic and nitrogen-sparing effects exerted by the mucin fraction used by us), different hypotheses may be advanced, viz.:

1) the action is mediated through a modification of the rat intestinal flora, perhaps related to the Bifidus factor activity of the blood group substances;7
2) the effect is a direct one on rat tissue metabolism, after the absorption of some decomposition products of the mucin fraction.

**Summary**

A mucin fraction with high content of A and H blood group substances was obtained from hog gastric mucosa. This fraction administered per os together with vitamin B$_{12}$ in rats made anemic by phenylhydrazine injection shows a significant effect on the hematologic response in comparison with the animal treated with vitamin B$_{12}$ alone.

The same fraction administered per os in cortisone-treated rats has also a significant nitrogen sparing action.

The possible mechanisms and relationships of the observed biologic effects are briefly discussed.
<table>
<thead>
<tr>
<th>Day on experiment</th>
<th>Group A Basal diet</th>
<th>Group B Basal diet, cortisol daily injected 20 mg./Kg.</th>
<th>Group C Basal diet, 0.3% of the mucin fraction, cortisol daily injected 20 mg./Kg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7th</td>
<td>10</td>
<td>161.9 ± 17.9</td>
<td>0.70 ± 0.009</td>
</tr>
<tr>
<td>8th</td>
<td>9</td>
<td>144.6 ± 25.7</td>
<td>0.70 ± 0.009</td>
</tr>
<tr>
<td>9th</td>
<td>8</td>
<td>141.5 ± 14.4</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>10th</td>
<td>9</td>
<td>138.4 ± 27.7</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>11th</td>
<td>9</td>
<td>158.4 ± 13.9</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td>All days</td>
<td>45</td>
<td>148.9 ± 21.9</td>
<td>0.72 ± 0.06</td>
</tr>
</tbody>
</table>
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SUMMARIO IN INTERLINGUA

Un fraction mucin a alte contento de substantias de gruppo sanguinee A e B esseva obtenite ab le mucosa gastric de porcos. Iste fraction, administrate oralmente in combination con vitamina B\textsubscript{12} a rattos que habeva essite facite anemic per injectiones de phenylhydrazina, exerce un effecto significative super le responsa hematologic in comparation con animales tractate con vitamina B\textsubscript{12} sol.

Le mesme fraction administrate oralmente a rattos tractate con cortisona effectua un augmento significative in le coefficiente de utilisation de nitrogeno.

Le mechanismos e le interrelationes possibile del observate effectos biologic es discutite brevemente.

REFERENCES


12. György, P.: Personal communication to the authors.


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