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METHAEMOGLOBINAEMIA AND COPROPORPHYRINURIA IN EXPERIMENTAL ANILINE POISONING

The authors have tried to establish on test animals the relations between the abnormal methaemoglobinaemia and the rise in coproporphyrinuria observed in aniline poisoning. Having observed that the rise of coproporphyrinuria is more pronounced when methaemoglobine had already in greater part left the circulating blood, they deny that there are any relations between the two pigments.

While the relations between haemoglobin and porphyrine have been studied by many workers, very little has been done so far to establish the relations of methaemoglobin and porphyrines. Brownelee (1933), Rimington, Hemmings (1939) and Keller (1939) observed a rise in the elimination of porphyrines in rats poisoned with various substances inducing the formation of methaemoglobin but, as some of these researchers gave contradictory results, Heubner (1940) denied that the formation of methaemoglobin may by itself cause a rise in the elimination of porphyrines. Seghini (1937) remarked that in aniline poisoned rabbits there appeared to be an evident rise of the urinary and fecal porphyrines. Similar results were obtained by Leroux (1947), who studied coproporphyrinuria in aniline poisoned men. Both Rimington and Brownelee suggest that the destruction of methaemoglobin takes place through a porphyrine III and not through bilirubine, as it is the case with haemoglobin, while Seghini, having observed in his tests that increased anaemia is accompanied by an increased content of bilirubinoid bodies, puts also porphyrinuria on account of the abnormal haemolysis. In none of these researches, however, determination of methaemoglobin was carried out. We think therefore that from these experiments it is not possible to obtain definitive indications as to the relation between these two pigments.
Vannotti (1948) described some cases of methaemoglobinæmia, one of which had a paroxysmic character (occasional appearance of methaemoglobin in blood with cyanosis without the presence of methaemoglobin producing substances) where the elimination of urinary porphyrines was normal, while in three cases of idiopathic methaemoglobinæmia a substantial increase of porphyrinuria was noted. In one of these cases determination of protoporphyrin in blood gave values not much above normal (10-15 μ g). Vannotti holds that, at least with regard to non-idiopathic methaemoglobinæmia cases, it should be possible to follow Rimington's point of view on the development of methaemoglobin and he suggests that the lack of catalase (to which, according to Heubner, the formation of methaemoglobin is to be related) may be caused by an alteration of the porphyrine metabolism; this lack of the ferment and of its activity should occur primarily in idiopathic forms so that there would not be any rise in porphyrinuria. In our opinion, there is not sufficient evidence for this view; one of us (Ghirlinghelli, 1952) has in fact observed that in experimental aniline poisoning blood values of catalase do not show any significant alterations while in lead poisoning, where there is, as already known, a pronounced alteration of the porphyrine metabolism, the methaemoglobin values are quite normal.

In our researches we have studied the behaviour of coproporphyrinuria and of methaemoglobinæmia in five test animals (dogs) poisoned by aniline in order to establish whether there is any relation between the two pigments.

The tests were made as follows: the animal was bound and catheterised; the urine excreted in a period of 8 hours was collected and aniline injected; the urines excreted in 8 hours following the aniline injection were separately collected; a third sample was obtained in the subsequent 8 hours. Coproporphyrine was determined in these three samples of urinoc by means of a method suggested by Vigliani. The aniline dosage was for three animals 10 mg per kilogram of weight; for the other two it was 20 mg per kilogram. Before the administration and 1, 1½, 2, 3 and 8 hours after administration of the poison the methaemoglobin was determined with Havemann's colorimeter using a method of Havemann, Jung and Issekutz (1938) modified by Angeleri (1949). In these conditions the methaemoglobin, formed in abnormal quantities inside the red cells, was being destroyed giving porphyrines, there would have to be a rise of coproporphyrinuria during the period of high levels of methaemoglobin in blood; moreover, it would have to be observed that the fall to normal blood level values was accompanied by a return of coproporphyrinuria to normal levels.

The results obtained are shown on table 1; the blood values of methaemoglobin refer to values found before poisoning, 8 hours after the administration of the poison and to the maximal values (which were found 1½ 2 hours after the aniline injection).

From the table it can be deduced that:
Table 1

<table>
<thead>
<tr>
<th>Nr. dogs</th>
<th>Weight</th>
<th>Aniline congo mg</th>
<th>Methaemoglobinemia [g/100 ml blood]</th>
<th>Coproporphyrinuria (in µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before poisoning</td>
<td>Maximal values</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0.18</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>16.9</td>
<td>10</td>
<td>0.14</td>
<td>2.92</td>
</tr>
<tr>
<td>3</td>
<td>11.5</td>
<td>10</td>
<td>0.15</td>
<td>2.92</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>20</td>
<td>0.15</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>13.2</td>
<td>20</td>
<td>0.21</td>
<td>7.12</td>
</tr>
</tbody>
</table>

(1) Methaemoglobinemia 8 hours after poisoning was nearly at same level as at the beginning of the test;

(2) Coproporphyrine detected in extremely small quantities in the urine samples taken before the test (average value: 1.85 µg) increased as shown by 2nd sample (average value: 5.9 µg) and particularly in the 3rd sample (average value: 7.5 µg), when the blood value of methaemoglobin was nearly back to normal;

(3) Both methaemoglobinemia and coproporphyrinuria were proportional to the amount of aniline injected.

Though, as can be seen from the present tests, the formation of methaemoglobin was followed by a rise of coproporphyrinuria, there does not appear to be any relation between two pigments. This follows from the following facts: (1) the rise of coproporphyrinuria has been more pronounced when methaemoglobin had already in greater part left the circulating blood and (2) it is not possible to maintain the assumption that the destruction of methaemoglobin may cause the formation of intermediate compounds which only after a given latent period, when methaemoglobin had nearly disappeared from the circulating blood, were transformed to porphyrines; it is well known, on the contrary, that the formation of porphyrines from the haematic pigment takes place very quickly both in vitro and in vivo.

The abnormal coproporphyrinuria observed in our test cannot therefore be put into relation with methaemoglobin, but with the accelerated building of fresh haemoglobin, due to the increased destruction of blood cells observed in aniline poisoning, and it should have a synthetic origin.

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SADRŽAJ

Autori su na eksperimentalnim životinjama otrovanima anilinom proučavali odnose između anormalne methemoglobinematije i povšenja koproproporfinurije. Budući da su opazili, da je povšenje koproproporfinurije jače izraženo, kada je methemoglobin velikim dijelom nestao iz cirkulirajuće krvi, autori zaključuju, da između spomenuta dva pigmenta ne postoji nikakva veza.

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