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R A D O V I

TRANSACTIONS ТРУДЫ

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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 haemoglobine and porphyrine have been studied by many workers, very little has been done so far to establish the relations of methaemoglobine and porphyrines. *Brownlee* (1933), *Rimington, Hemmings* (1939) and *Koller* (1939) observed a rise in the elimination of porphyrines in rats poisoned with various substances inducing the formation of methaemoglobine but, as some of these researches gave contradictory results, *Heubner* (1940) denied that the formation of methaemoglobine 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 Brownlee suggest that the destruction of methaemoglobine takes place through a porphyrine III and not through bilirubine, as it is the case with haemoglobine, 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 methaemoglobine 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 methaemoglobinaemia, one of which had a paroxysmic character (occasional appearance of methaemoglobine in blood with cyanosis without the presence of methaemoglobine producing substances) where the elimination of urinary porphyrines was normal, while in three cases of idiopathic methaemoglobinaemia a substantial increase of porphyrinuria was noted. In one of these cases determination of protoporphyrine in blood gave values not much above normal (10–15 μ g). Vannotti holds that, at least with regard to non-idiopathic methaemoglobinaemia cases, it should be possible to follow Rimington's point of view on the development of methaemoglobine and he suggests that the lack of catalase (to which, according to Heubner, the formation of methaemoglobine 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 (*Ghiringhelli*, 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 methaemoglobine values are quite normal.

In our researches we have studied the behaviour of coproporphyrinuria and of methaemoglobinaemia 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 separatelly collected; a third sample was obtained in the subsequent 8 hours. Coproporphyrine was determined in these three samples of urine 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, 1$^1/_2$, 2, 3 and 8 hours after administration of the poison the methaemoglobine was determined with Havemann's colorimeter using a method of *Havemann, Jung* and *Issecutz* (1938) modified by *Angeleri* (1949). In these conditions the methaemoglobine, 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 methaemoglobine 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 methaemoglobine refer to values found before poisoning, 8 hours after the administration of the poison and to the maximal values (wich were found 1$^1/_2$ 2 hours after the aniline injection).

From the table it can be deduced that:

Table 1

Nr. dogs	Weight kg	Aniline dosage mg/kg	Methaemoglobinaemia (g/100 ml blood)			Coproporphyrinuria (in μg)		
			Before poisoning	Maximal values	8 hours after injection	1st sample	2nd sample	3rd sample
1	10	10	0.18	3	0.30	traces	3.55	5.25
2	16.9	10	0.24	2.80	0.57	2.25	6.50	7
3	11.5	10	0.21	2.92	0.62	3	5.25	6.20
4	12	20	0.15	8	0.85	2	7.25	8.75
5	13.2	20	0.21	7.12	0.92	2	7	9.25

(1) Methaemoglobinaemia 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 methaemoglobine was nearly back to normal;

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

Though, as can be seen from the present tests, the formation of methaemoglobine 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 methaemoglobine had already in greater part left the circulating blood and (2) it is not possible to maintain the assumption that the destruction of methaemoglobine may cause the formation of intermediate compounds which only after a given latent period, when methaemoglobine 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 methaemoglobine, but with the accelerated building of fresh haemoglobine, 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 methemoglobinemije i povišenja koproporfirinurije. Budući da su opazili, da je povišenje koproporfirinurije 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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