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PARTICULAR FEATURES OF PORPHOBILINOGEN SYNTHESIS FROM δ-AMINOLEVULINIC ACID IN VISCERAL TISSUES OF RATS

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KEY WORDS: lead poisoning; porphobilinogen; aminolevulinic acid.

The biosynthesis of porphobilinogen, a precursor of heme, begins with a reaction of condensation of two δ-aminolevulinic acid (ALA) molecules, catalyzed by ALA dehydratase. The most intensive formation of the porphyrin takes place in bone marrow cells, followed by liver and, at a lower level, the remaining organs of animals [2, 9].

Activity of ALA dehydratase is inhibited by lead by a noncompetitive mechanism [13]. ALA dehydratase of erythrocytes is the most sensitive to the inhibitory action of lead [10]; the enzyme of the liver and kidneys [7, 14] and of other viscera [11] is less sensitive.

This paper describes a comparative study of porphobilinogen biosynthesis from ALA by homogenates of rat visceral tissues, using administration of lead as a method of revealing the particular features of porphyrin formation in different organs.

EXPERIMENTAL METHOD

Experiments were carried out on 145 noninbred male albino rats weighing 260-310 g, of which 77 animals were controls and 68 were poisoned with lead acetate by subcutaneous injection in a dose of 25 mg lead/kg body weight. The poisoning continued for 5-6 weeks, with three injections a week. Control rats were given injections of distilled water.

The development of lead poisoning was judged from the general condition of the animals, changes in the red blood picture, and disturbance of porphyrin metabolism. The hemoglobin level and reticulocyte count were determined in the usual way, and the number of erythrocytes with punctate basophilia was counted after "enrichment" in a moist chamber. The ALA concentration in the urine was determined by the method in [12] in the writers' own modification [3]. Iron in the plasma was determined by the orthophenanthroline method. The rats were killed by decapitation.

ALA dehydratase activity was estimated from the quantity of porphobilinogen (in μg/mg protein/2 h of incubation) synthesized by visceral homogenates from a 0.1 M solution of ALA added [1, 4]. Protein was determined by Lowry's method. Squash preparations from the liver, kidneys, and spleen for cytological investigation were stained with azure-eosin.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that in the rats receiving lead acetate there was a tendency for body weight to fall and anemia to develop, accompanied by an increase in the number of reticulocytes and stippled erythrocytes and a marked increase in the ALA concentration in the urine. These observations indicated the development of severe lead poisoning in the rats. The plasma iron level showed no significant change under these circumstances.

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TABLE 1. Principal Indices Characterizing Development of Lead Poisoning in Rats (M ± m)

Index	Rats receiving lead acetate		Rats receiving placebo (control)	
	before beginning of injections	before sacrifice	before beginning of injections	before sacrifice
Body weight, g	302,7±5,24	288,36±6,49	305±10,3	335,9±18,3
Hemoglobin concentration, g%	13,1±0,076	7,8±0,18	12,6±0,24	12,3±18,3
Reticulocyte count, %	2,5±0,15	9,9±0,8	2,5±0,15	3,65±0,39
Number of stippled erythrocytes per 10,000 cells	1,7±0,46	34,2±0,76	1,7±0,46	1,6±0,42
ALA in urine, mg%	0,29±0,03	11,31±0,95	0,29±0,03	—
Plasma iron concentration, μg/100 ml	207±5,3	214±3,2	—	—

The layer of fat on these animals was absent or considerably reduced, and the weights of the spleen, liver, and kidneys were increased. For instance, whereas in intact rats the spleen weighed 0.7 ± 0.02 g, in rats receiving subcutaneous injections of distilled water it weighed 1.27 ± 0.12 g, and in rats receiving injections of lead acetate, it weighed 2.1 ± 0.1 g.

Changes in ALA dehydratase activity in rats with lead poisoning varied in different experiments. In homogenates of the lungs, kidneys, and heart ALA dehydratase activity was reduced: in the lung tissues by 40.3% (from 3.62 ± 0.35 to 2.16 ± 0.17 μg/mg; $P < 0.01$); in the kidneys by 40% (from 4.31 ± 0.25 to 2.59 ± 0.19 μg/mg; $P < 0.01$), and in the heart by 38.2% (from 2.7 ± 0.49 to 1.67 ± 0.14 μg/mg; $P < 0.05$). Activity of the enzyme in the tissues of the liver, pancreas, and small intestine was unchanged, and in the spleen it was increased by 26.3% – from 2.47 ± 0.2 to 3.12 ± 0.23 μg/mg protein 2 h of incubation ($P < 0.05$). By analogy with anemia in other pathological conditions, the compensatory development of foci of extramedullary hematopoieses in certain organs with high ability to synthesize heme in normoblasts may be postulated [1, 5].

In squash preparations of the spleen from intact rats most cells were lymphocytes, predominantly small lymphocytes (93.5%), and a few cells could be classed as prolymphocytes. The spleen cell composition of some rats included solitary plasma cells, macrophages, and mast cells as well as a few cells representative of bone marrow hematopoiesis – myelocytes, metamyelocytes (between 0.04 and 0.09%); normoblasts numbering 0.4 ± 0.13% were found in all rats.

In the spleen cell composition of rats with lead anemia, against the background of a predominance of lymphocytes there was a considerable increase in the number of normoblasts, mainly on account of polychromatophilic and oxyphilic forms, which amounted on average to 20.5 ± 1.2%. Only in one case, in which the percentage of normoblasts was particularly high (28.8%) were many basophilic forms found. In some preparations megakaryocytes were seen, evidence of the development of foci of myeloid hematopoiesis in the spleen.

Cytological examination of squash preparations of the liver and kidneys of the rats with lead poisoning revealed single normoblasts in preparations from one-third of the animals against the background of hepatocytes and renal epithelium respectively and of cell debris.

The increase in ALA dehydratase activity in the spleen tissue can evidently be explained by the appearance of cells of ectopic hematopoiesis in it.

The intensity of porphobilinogen synthesis thus differed in different organs. The sensitivity of the enzyme which catalyzes this process in the organs to the action of inhibitors, especially lead, also differed: in some organs a decrease in activity of the enzyme was observed, in others its activity was unchanged, and in some (the spleen) it was increased. This may be attributable to the presence of isozymes of ALA dehydratase. The increase in enzyme activity in the spleen was evidently due to the development of foci of ectopic hematopoiesis in that organ.

It is an interesting fact that changes in ALA dehydratase activity in the same organs varied when lead was given in vivo or added in vitro [2]. Similar data are to be found in the literature on UDP-bilirubin-glucuronyl transferase and γ-glutamyl transpeptidase [8], and mixed oxidases [15]. In the whole organism, lead may perhaps not only affect biosynthesis of hemoproteins, but may also act on their breakdown [15].

Besides the acting dose, the root of entry of lead into the body – peroral or parenteral – also is important [6].

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LIVER ORGAN CULTURE FROM SUCKLINGS BORN TO MICE INFECTED WITH COXSACKIE VIRUS DURING PREGNANCY

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KEY WORDS: Coxsackie A13 virus; organ culture; proliferation; liver.

The writers previously established the marked cytoproliferative action of Coxsackie A13 virus during organ culture of liver obtained from females infected once with the virus during pregnancy [4]. Since manifestations of the cytoproliferative effect in experiments in vivo and in vitro are characteristic of chronic virus infection [1], the object of this part of the investigation was to study growth of an organ culture of liver taken from the young mice born to these mothers.

Experiments were carried out on noninbred albino mice weighing 20-22 g. The mothers were infected with undiluted virus-containing culture fluid, containing $10^{5.35}$ TCD_{50}/ml. The virus was injected intramuscularly into the animals once, on the 7th day of pregnancy, in a dose of 1 ml. The day of discovery of a vaginal plug was taken as the first day of pregnancy.

Eight sucklings divided into two groups were used in the experiment: group 1 (experimental) consisted of five newborn mice autopsied 15 days after injection of virus into the mothers; group 2 (control) consisted of three sucklings obtained from uninfected healthy mothers. Virus was isolated from the liver tissue of the experimental newborn mice by the usual methods.

Organ culture of the liver was carried out by Grobstein's method in the modification of Luriya and P'yanchenko [3], in Conway dishes at the partition boundary between two media. Details of the method were described previously [4].

Altogether 88 explants were studied. To compare growth of organ cultures of the liver from mice of the above groups, features reflecting the character of growth of cells around the explant (absence of growth, small zone of cell growth, good growth — a wide zone of cell growth), the presence of lymphocytes in the zone of

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