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Effects of Iron-Loading and Ethanol Treatment on Rat Porphyrin Metabolism

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KÓSZÓ, F., CS. SIKLÓSI AND N. SIMON. *Effects of iron-loading and ethanol treatment on rat porphyrin metabolism.* PHARMAC. BIOCHEM. BEHAV. 13(3) 325–329, 1980.—Rats were fed for 50 weeks with a standard diet containing 5.9% ferric ammonium sulphate. Half of these animals drank normal water, and the other half water containing 5% ethanol (groups 1 and 2). Two other groups received normal food, but drank water containing 5 or 10% ethanol (groups 3 and 4) for 40 weeks. Histologic examinations revealed that the iron-loading resulted in only mild hepatic siderosis in groups 1 and 2, the degree of siderosis not differing appreciably in the two groups. The ethanol led to fatty degeneration too in the liver of animals in group 2. Both iron-loading and ethanol treatment, either separately or in combination, increased the porphyrin excretion, but the distribution of the various porphyrins in the urine and faeces showed merely the symptoms of an aspecific poisoning. A significantly elevated uroporphyrin excretion was not observed in any of the groups, and thus the results support the view that dietary iron-loading and ethanol consumption can not be regarded as direct aetiologic factors in the pathomechanism of porphyria cutanea tarda. At the same time, the results suggest that vitamin E therapy, frequently employed effectively in porphyria cutanea tarda, can not be considered a causal intervention as regards the mechanism of action.

Iron-loading Ethanol Porphyrin metabolism Vitamin E Rats

OF the various porphyria forms, perhaps the pathogenesis of porphyria cutanea tarda (PCT) involves most open questions. It appears that the disease is basically hereditary, following the rules of polygenetic inheritance, and that hepatotoxic factors play a role in the manifestation of the disease [30]. It is important, therefore, to study how the occurrence of the disease is influenced by dietary and environmental factors and by different drugs. The biosynthesis of haeme is known to require iron, and thus it is logical to assume that an iron-metabolism disturbance will affect the biosynthesis of haeme. This seems to be supported by the hepatic siderosis and the increased total iron content of the organism in PCT patients [2, 11, 16, 19, 28, 31, 36], and by the changes in the amount of stored iron accompanying phlebotomy, currently considered the most effective therapy [7, 17, 20, 26]. The relatively long-studied role of ethanol [8] also appears obvious; its direct ALA-synthetase-inducing effect [29], its ALA-dehydratase-depressing effect [21], and its indirect effect via the iron metabolism [3, 18, 33] have been proved. Even now, however, we do not know the exact, detailed mechanism of action whereby a role is played in the pathomechanism of PCT and tarda-like porphyrias by iron and ethanol, which individually are involved in many very diverse interactions with other diseases too, either connected with the porphyrias or independent of them (e.g. deficiency diseases, anaemias, hepatic diseases, etc.).

In order to obtain further data relating to the above question, we have studied the direct effects of chronic iron-loading and of ethanol treatment on the porphyrin metabolism in rats.

METHOD

Animals; Chemicals

Young male white rats of the CFY strain, with initial body weights of 150–200 g, were used. All chemicals were REANAL products of analytical purity.

Iron-Loading; Ethanol Treatment

Group 1: 40 rats were fed for 50 weeks with a diet containing 5.9% ferric ammonium sulphate, $FeNH_4/SO_4/_2 . H_2O$. This corresponded to a daily consumption of about 10 mg iron per animal. Group 2: After the 7th week, 20 animals from group 1 were given drinking-water containing 5% v/v ethanol, the iron diet being maintained. Groups 3 and 4: Two groups of 20 rats each were fed for 40 weeks with normal food, but their drinking-water contained 5 or 10% v/v ethanol; this corresponded to a daily consumption of approximately 1 or 2 ml pure ethanol, respectively. Apart from the drinking-waters, both diets (groups 1 and 2 vs groups 3 and 4) were identical except for the ferric ammonium sulphate. In the course of these chronic poisonings, the four animal groups consumed the appropriate diets ad lib.

Assays

During treatment, the animals were placed in individual

Abbreviations:
PCT: porphyria cutanea tarda
ALA-synthetase: δ-aminolevulinic acid synthetase
ALA-dehydratase: δ-aminolevulinic acid dehydratase
GSH: reduced glutathione
GSSG: oxidized glutathione
NADH: reduced nicotinamide-adenine dinucleotide
NAD: nicotinamide-adenine dinucleotide

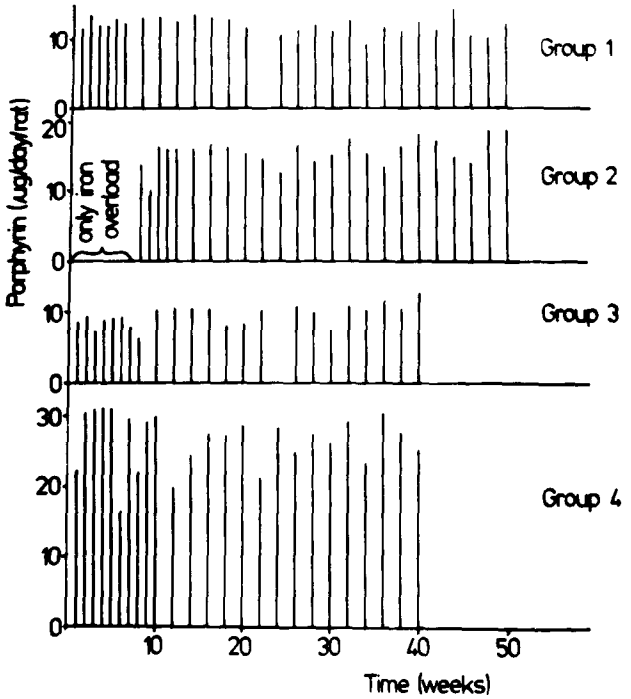

FIG. 1. Urinary total porphyrin excretion in the four groups of rats. Group 1: iron-loading; group 2: iron-loading and 5% ethanol; group 3: 5% ethanol; group 4: 10% ethanol treatment.

cages, at first weekly but at fortnightly intervals after the 10th week, for collection of the 24-hour urine and faeces. The total porphyrin contents of the excreta were determined separately on each individual sample, while the distribution of the various porphyrin fractions was generally obtained by the analysis of group samples resulting from the combination of samples with known total porphyrin contents. Urinary and faecal porphyrins were determined by the method of Doss [6], the faecal samples being extracted on the basis of the procedure of Heller *et al.* [10]. The porphobilinogen content of the urine was tested according to Watson and Schwartz [37].

Histology

On completion of the experiments, 2 animals each from groups 1 and 2 and from an untreated control group were sacrificed for histologic examination of their livers. Sections prepared from samples embedded in paraffin were stained with haematoxylin-eosin or with Prussian blue, while sections originating from frozen samples were stained with Oil red.

RESULTS

Around the 5th week of treatment enhanced restlessness was observed in the animals of group 1, with later a mild tremor. Their white hair turned brownish, their faeces was markedly tarry, and their urine varied in colour from light- to dark-brown. On continuation of the iron-loading, however, the general condition of the animals did not deteriorate, but after the 10th–15th week slowly improved: the restlessness and tremor progressively diminished, and then disappeared. The joint administration of ethanol and iron in group 2 led to the same but no additional external effects. No external signs

TABLE 1

ANALYSIS OF VARIANCE FOR 2-WAY CLASSIFICATION WITH MISSING DATA

Urinary total porphyrin:	*Groups 1, 2, 3, 4, 5 not differ, $p=0.359$
Urinary coproporphyrin:	Groups 1, 2, 3, 4 differ, $p<0.05$
Urinary uroporphyrin:	Groups 1, 2, 3, 4 not differ, $p=0.114$
Urinary + fecal total porphyrin:	Groups 1, 2, 3, 4, 5 differ, $p<0.05$

*1, 2, 3, 4: treated groups.
5: untreated control group.

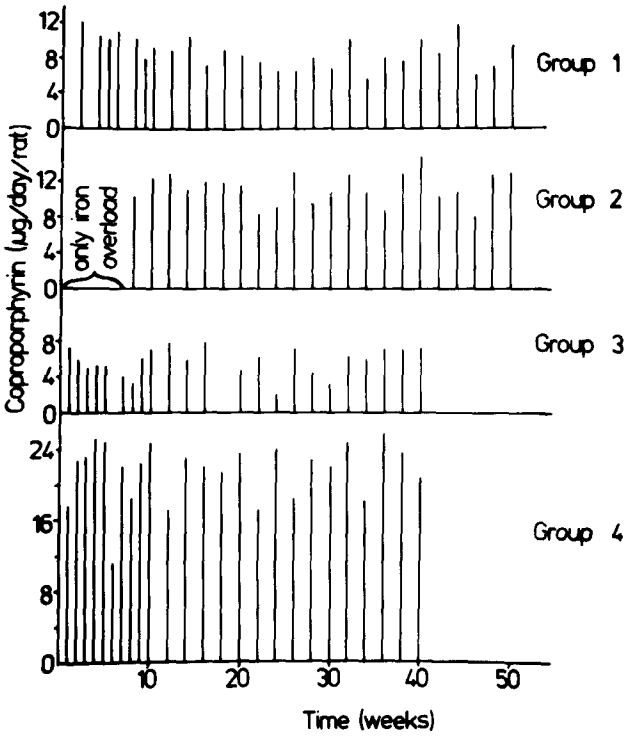

FIG. 2. Urinary coproporphyrin excretion.

at all were observed in group 3, where the water contained 5% ethanol. Initially, the animals in group 4 were unwilling to accept the 10% ethanol solution, and consumed a normal quantity of liquid only after 2–3 weeks.

From the first week the porphyrin excreted in the urine was mildly elevated in all four groups (Fig. 1). A rapid increase occurred only in group 4, however, where the daily excretion was four times that of the untreated control group, the maximum being attained in the 4th week: 31.55 ± 7.23 µg/day/rat. Although urinary porphyrin level in all groups exceeded that of the untreated control at every date of the experiment, according to analysis of variance the groups did not differ (Table 1). During treatment, an enhanced porphobilinogen excretion was not found in the urine of any of the groups.

Chromatography and measurement of the various porphyrins in the urine during treatment showed a predomi-

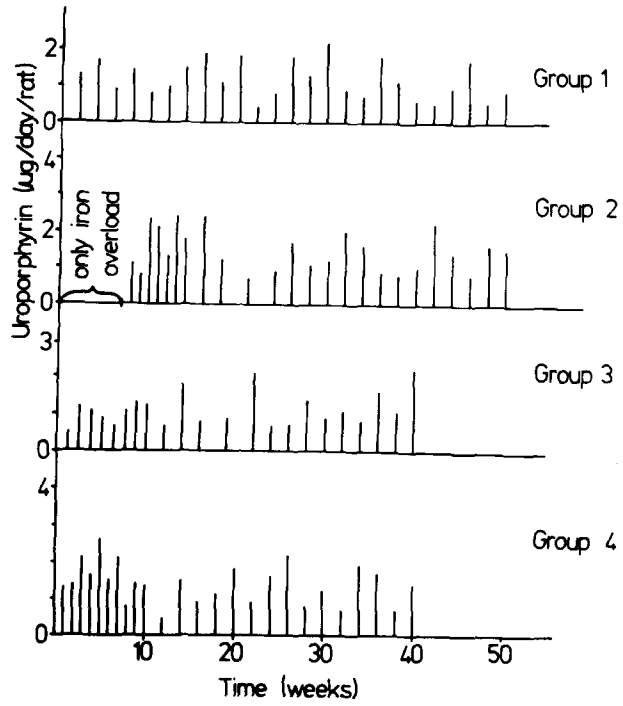

FIG. 3. Urinary uroporphyrin excretion.

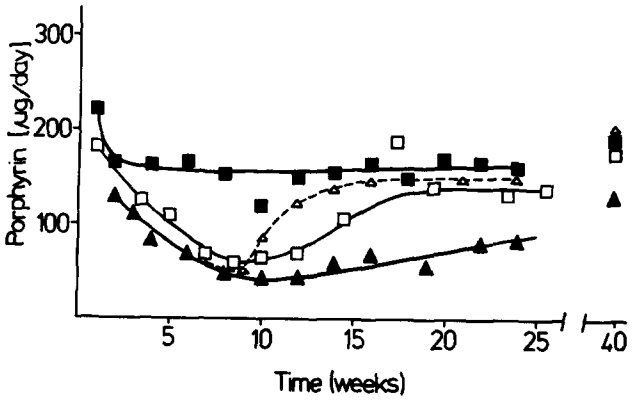

FIG. 4. Urinary and fecal total porphyrin excretion in consequence of the different treatments: ▲ iron-loading; △ iron-loading + 5% ethanol; □ 5% ethanol; ■ 10% ethanol.

nance of coproporphyrin in every group. The uroprophyrin excretion transitionally increased a little in groups 2 and 4 around the 4th–5th week of the poisoning, but even when the animals excreting most total porphyrin were tested individually, the highest uroporphyrin values did not exceed 5 µg/day. The copro- and uroporphyrin excretions of the groups are depicted in Figs. 2 and 3. Apart from slight and temporary changes in the 5th–15th weeks, the ratios of the different porphyrins did not vary essentially in either the urine or the faeces during the chronic treatment. The data for the 40th week are tabulated (Table 2). It is noteworthy that analysis of variance revealed difference only in respect of the urinary coproporphyrin excretion, however, the groups did not differ in respect of the urinary uroporphyrin excretion (Table 1). As to urinary coproporphyrin, all the groups differed significantly from each other (at the level of 95%) especially the group 4.

TABLE 2

PORPHYRIN EXCRETION [µg/day/animal] IN THE FOUR GROUPS OF RATS AT 40th WEEK OF TREATMENT

Porphyrins		Groups*			
		1	2	3	4
Urinary	proto	0.60	1.08	1.30	1.80
	3-COOH	0.18	0.12	0.09	0.35
	copro	9.92	14.92	7.08	20.36
	5-COOH	0.20	0.36	0.52	0.63
	6-COOH	0.14	0.30	0.47	0.20
	7-COOH	0.18	0.68	1.10	0.69
	uro	0.59	0.97	2.21	1.35
	total	11.81	19.15	12.77	25.38
Fecal	proto	56.00	99.13	103.50	112.97
	copro	48.50	57.40	47.80	36.32
	total†	115.50	172.68	164.30	161.44

*Group 1: iron-loading.
Group 2: iron-loading + 5% alcohol.
Group 3: 5% alcohol.
Group 4: 10% alcohol.
†Similarly as for the urinary porphyrins, fecal total porphyrins contain also other porphyrins of different number of carboxyl groups.

Figure 4 shows the changes in the total porphyrins excreted in the urine and the faeces. In every group a temporary fall occurred after the initial high values. In group 2 the commencement of ethanol treatment on top of the iron-loading caused a sudden rise in the daily total porphyrin output. In the other groups the excretion levels slowly approached one another in the second halves of the curves (roughly after the 15th week) and attained saturation values (at different times, but finally at approximately the same level). According to analysis of variance the behaviour of the groups were different (Table 1). As to Scheffé test, the excretion in group 1 proved to be significantly lower than that of the others from week 10 to week 40. There was a more considerable difference between group 4 and the other three groups from week 5 to week 10. After the 7th week, iron-loading combined with alcohol treatment resulted in significantly higher porphyrin excretion than iron-loading alone. According to analysis of variance for 2-way classification without interaction the significant difference in the case of the treated groups was 10.2404 (at 95%). The different behaviour of the groups can be mainly explained by the dissimilarities in the amounts of fecal porphyrins.

Histologic examination with haematoxylin-eosin staining in group 1 revealed a moderate degree of degenerative change in the plasma, a little mononuclear, eosinophilic granulomatous infiltration in the portal areas, and mild siderosis in the stellate cells. Haemosiderin was observed in the cytoplasm of the hepatocytes with the Prussian blue reaction. Similarly as for the untreated control, Oil red gave a negative picture. In group 2 haematoxylin-eosin staining demonstrated a fatty, patchy degeneration in the plasma. Similarly as in group 1, a moderate amount of haemosiderin was detected in the cytoplasm with the Prussian blue reaction, while Oil red sections showed patchy, lipid droplet degeneration. Only mild hepatic siderosis was observed in the two groups, therefore, which did not differ perceptibly in the degree of siderosis.

DISCUSSION

In non-porphyric individuals ethanol increases the excretion of coproporphyrin, but not that of uroporphyrin [8, 24, 34]. This effect can be attributed to the property of ethanol in enhancing the ALA-synthetase activity [29]. It is a general experience that the large majority of PCT and symptomatic porphyria patients at the same time overindulge in alcohol. This justifies further investigations in connection with ethanol. Our present experiments indicated that ethanol consumption by the animals (independently of the concentration employed) resulted merely in the classic symptoms of a poisoning: predominantly coproporphyrin was excreted in the urine, and proto- and coproporphyrin in the faeces. In agreement with the findings of other authors relating to a shorter treatment period [12,25], we did not find an appreciable uroporphyrin excretion in response to this long-lasting treatment either. Accordingly, we do not regard ethanol as a direct aetiologic factor in the development of PCT. However, as confirmed by the present experiments and by our observations on humans, ethanol undoubtedly plays a role in precipitating and exacerbating PCT, both via its direct inductor effect on porphyrin synthesis and via its indirect effect assumed on the basis of liver damage.

The duration and concentration of the iron-loading employed in our experiments were far in excess of those possible under natural conditions. This situation was further enhanced by the simultaneous administration of ethanol, which indirectly increases the absorption of ferric iron [3,18]. Nevertheless, even lower extents of hepatic siderosis and uroporphyrin excretion were observed than those reported by Shanley [29]; we ascribe this to the different feed composition. However, it is necessary to reckon with the fact that the total body iron, and within this the hepatic iron concentration, are certainly elevated [29]. A high dose of ferric citrate is known to have a synergistic effect on the induction of hepatic ALA-synthetase [31]. The fact that in spite of this a significantly enhanced porphyrin excretion was not found in our experiments is explained by the circumstance that the iron at the same time increases the degradation of haeme and haeme compounds too [4,5]. In our experiments the intensified turnover of haeme compounds is indicated by the very strong positivity of the chromatographic plates towards the benzidine reagent in every case.

Kushner *et al.* [13] report that iron inhibits uroporphyrinogen-decarboxylase. Only the decrease of uroporphyrinogen-decarboxylase activity has been demonstrated as a specific enzyme defect in PCT [14,15]. If this enzyme defect itself were sufficient to explain the predominant uroporphyrin excretion characteristic of PCT, we might expect a higher uroporphyrin excretion following the administration of iron. This was not observed in our experiments, however, though it is true that the diet employed resulted in only a relatively mild degree of siderosis in the animals.

In an assessment of the role of iron, some other factors too must be considered. Gillman *et al.* [9] and Shanley *et al.* [29] used white maize meal as deficient diet and gave the rats milk supplementation in order to obviate rapid deterioration of the animals. The deficient diet in their experiments had a complex meaning involving Ca-, P-, vitamin- and amino acid deficience. On the basis of their observations, since experimental hepatic siderosis can be induced more easily with an iron-rich but deficient diet than with an iron-rich normal diet, the effect of the feeding conditions is always combined with the direct effect of iron-loading. Further data are necessary to establish the connections between iron-loading and hepatic siderosis and the secondary porphyrias observed in the various deficiency diseases (pellagra, pellagroid). All the same, in the knowledge of the present experimental results and the above literature data, much greater importance must be attached to the feeding conditions as regards the development of hepatic siderosis in PCT cases. Attention must be paid to the fact that the application of vitamin E therapy in PCT has sometimes proved effective [22,23]. As a potential antioxidant, vitamin E may be considered a therapeutic the deficiency of which can be brought into a causal connection with the premature oxidation of uroporphyrinogen to uroporphyrin [1]. This view of the redox conditions appears to be supported somewhat by the NAD/NADH or GSSG/GSH ratio shifts found in experimental porphyrias [27,35]. Accordingly, therefore, a porphyrin excretion reminiscent of PCT should be observed in a vitamin E-deficient state. Because of the oxidizing ability of ferric iron, the very high concentration of ferric iron in the diet used in this experiment must also have resulted in a vitamin E-deficient state in the animals, but a noteworthy uroporphyrin excretion was nevertheless not observed. In this respect our experimental results indirectly argue against the causal therapeutic effect of vitamin E.

For light to be shed on the reasons for the pathologic uroporphyrin excretion characteristic of PCT, we feel that it would be very promising to carry out investigations of the permeability of the hepatic mitochondrium membranes, in which the transport conditons across the membrane and the membrane potential changes could be studied by taking into account not only the redox conditions but also the non-energy-dependent transport.

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