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THE ROLE OF POSSIBLE MEMBRANE DAMAGE IN PORPHYRIA CUTANEA TARDA: A SPIN LABEL STUDY OF RAT LIVER CELL MEMBRANES

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Abstract—Two groups of rats were made porphyric by treatment for a shorter or a longer time with hexachlorobenzene (HCB); a third group was subjected to chronic treatment with ethanol; the fourth group comprised untreated, control animals. Suspensions were prepared of hepatocytes isolated from the livers of the animals in the individual groups, and in these systems the cell membranes were studied by spin labeling. In all three chronically treated groups, the order parameter calculated from the ESR spectra was significantly lower than that of the control group. The order parameters for the first three treated groups, however, did not differ significantly from one another. If either HCB or ethanol was incorporated into the isolated hepatocytes of the healthy control animals, the fluidity of the hepatocyte membranes increased. The order parameter decrease (compared to the control) for the hepatocyte membranes of the animals treated chronically with HCB or ethanol can be ascribed to the direct membrane-fluidizing effect of HCB or ethanol, and also to the altered lipid metabolism. With regard to the difference in the mechanisms of action of HCB and ethanol, on the basis of the experimentally proved membrane-damaging effect of the porphyrinogenic HCB it is probable that, besides other factors, the membrane damage may play an important role in the pathogenesis of porphyria cutanea tarda.

Each of the different types of porphyrin metabolism disturbance is manifested by the excretion of certain precursors or porphyrins, produced as a consequence of well-defined enzyme deficiencies. As regards a characteristic enzyme deficiency in the case of porphyria cutanea tarda (PCT)†, however, to date it has only proved possible to demonstrate a reduced activity of uroporphyrinogen decarboxylase (EC 4.1.1.37) (urogen-D) in the liver [1–3] and in the erythrocytes [1, 4–6]. The change in activity of this enzyme corresponds theoretically to the observed biochemical finding: a pathologically enhanced uroporphyrin excretion; indeed it appears that the disease is of hereditary nature by virtue of the decreased urogen-D activity [1, 4, 5]. Nevertheless, a number of factors indicate that this decreased activity is in itself not sufficient to explain the pathomechanism of PCT. Examples of such factors: the porphyrin excretion by PCT patients is not in a close correlation with the extent of the observed enzyme deficiency; a decrease in the erythrocyte urogen-D activity can frequently be demonstrated in the members of the patients' families, but unaccompanied by pathologic porphyrin excretion [1]. These apparent contradictions are largely resolved by the work of Elder *et al.* [7], who suggest that there are probably two types of PCT: in one type, both the hepatic and the erythrocyte urogen-D activity is decreased, while in the other the activity in the erythrocytes is normal. However, the questions remain open as to the nature of the factors which determine which type of the disease will develop, when, and how. The different character of this clinical picture is further indicated by the fact that, whereas the other porphyria forms are acute, PCT is chronic in nature; and though the disease is considered to be fundamentally an inherited one [8], certain compounds, such as hexachlorobenzene (HCB) or 2,3,7,8-tetrachlorodibenz-*p*-dioxine, can nevertheless induce toxic porphyria in humans too, with clinical and biochemical symptoms corresponding to those of PCT [9].

In connection with the mechanism of development of PCT, we too earlier made investigations on the generally-used HCB-induced experimental porphyria in rats, and came to the conclusion that, besides the specific enzyme deficiency in PCT, i.e. the reduced urogen-D activity, not only is the regulation of porphyrin synthesis impaired at the chromosomal level, but there must also be another factor, namely the damage to the membranes of the organelles, which (together with other factors) may be responsible for the enhanced uroporphyrin excretion and for the chronic nature of the disease. With a view to testing the correctness of our hypothesis, we set out to obtain direct evidence regarding the existence (or non-existence) of HCB-induced membrane damage, by using a spin labeling technique to study the lipid membranes in isolated rat hepatocyte systems.

MATERIALS AND METHODS

Treatment of animals; porphyrin analyses

All rats used were males of the CFY strain, of the same age, with an initial body weight of 350–400 g. Group 1: 20 rats were fed for 11 weeks with feed containing 0.2% (w/w) HCB. Group 2: 10 rats were made strongly porphyric by treatment with HCB for 2.5 months; they next received normal feed transi-

† Abbreviations: HCB, hexachlorobenzene; PCT, porphyria cutanea tarda; urogen-D, uroporphyrinogen decarboxylase.

tionally, for 4 months; finally, they were again given feed containing 0.2% (w/w) HCB for 11 weeks. Group 3: 10 rats maintained on normal feed received drinking-water containing 10% (v/v) ethanol for 11 weeks. Group 4: 15 untreated animals.

With the aim of observing any markedly different behaviour from the average, in week 10 of treatment porphyrin analyses were performed on 24-hr urine and faeces samples collected simultaneously from 5 animals in each group. Subsequently, in combination with samples with known total porphyrin contents, the distributions of the different porphyrins were determined for each group. The method of Doss [12] was used for porphyrin analysis.

For examination of the liver, in group 1 one animal was sacrificed weekly from the end of week 6, and two animals in the final week (a total of 7); in group 2 one animal was sacrificed weekly from the end of week 7 (a total of 5); and in group 3 one animal was sacrificed weekly from the end of week 8 (a total of 4).

Unless otherwise stated, the chemicals used were REANAL products of analytical purity.

Preparation of isolated hepatocytes

Pieces weighing about 0.5 g from the left lobe of the liver of one animal in each group were cut into thin slices under physiological saline solution, the blood was well washed out with physiological saline, and then, at 37°, with constant shaking, the slices were treated with 5 ml portions of 0.5% (w/w) trypsin solution in physiological saline. Each digestion period lasted about 3 min. The dilute cell suspension portions obtained in each step were poured off the liver slices and collected in a 0° water-bath. A total of about 100 ml trypsin solution was employed for each sample. The combined hepatocyte suspensions were washed 4 times with physiological saline cooled to 4° (by centrifugation at low rpm, and resuspension) to purify the systems of the residual trypsin and the small amounts of cell debris. The pure hepatocyte suspensions were finally adjusted to a concentration of 1×10^8 cells/ml, with cell counting in a Bürker chamber by means of a microscope. These suspensions, with a volume of about 1 ml, were ample for the subsequent examinations. The samples were stored at +4° until the ESR measurements on the following day. In the interest of a reliable comparison, in the course of the preparation the samples from the different groups were treated simultaneously and in strictly the same manner, from every aspect.

Preparation of cell suspensions for spin labeling experiments

One μl of a 0.1 mg/ml stock solution of rotenone in ethanol was added to 100 μl of the 10^8 cells/ml suspension and vigorously mixed with a vortex mixer. Rotenone, a respiratory chain inhibitor, was needed to eliminate inherent fast signal reduction. A 2 μl aliquot of a 5 mg/ml solution of 5-doxyl stearic acid (Syva, U.S.A.) in ethanol was also added subsequent to rotenone inhibition. As judged from the spectral parameters (see below), samples prepared with the above procedure were magnetically dilute.

For measurement of the fluidizing effect of HCB, 2–50 μl portions of a 1 mg/ml solution of HCB in chloroform were layered onto the bottom of glass tubes and evaporated under N_2 to dryness. After addition of the cell suspension, the spin-labeling procedure was identical to that described above, except that the ethanol content of the sample solution was adjusted to 5% (v/v) to facilitate HCB membrane incorporation. In ethanol-fluidizing experiments, increasing amounts of ethanol were added to the spin-labeled cell suspension and viability checks were made by visual inspection under a microscope.

ESR measurements

ESR spectra were recorded with a JEOL (Japan) JES-PE-1X X-band spectrometer, using 100 kHz modulation technique. The cell suspension was added to a micro flat cell (Scanlon, U.S.A.), which was then sealed, and the spectrum was measured at room temperature. The order parameter was calculated from the splitting of the outer (A_{max}) and inner (A_{min}) maxima after $A \perp$ and polarity correction according to Griffith and Jost [13]:

$$S = \frac{A_{\parallel} - A\perp}{26.3\ G} \cdot \frac{a_0}{a}$$

where a and a_0 denote the measured values of the hyperfine tensorial trace and some arbitrary reference/a_0 = 44.6 G/. The effect of line-broadening due to probe–probe interaction was quantitated by introducing the m-parameter (Sauerheber *et al.* [14]). With careful dosing of the spin probe, $m \geq 0.95$ could be adjusted in each case, i.e. the samples were magnetically dilute.

RESULTS

The porphyrin excretions of the animals in the various groups are detailed in Table 1. In week 10 of HCB poisoning, every animal in group 1 was already in a very severe porphyric state: the total porphyrin excretion in the urine was 450–580 μg/day, with an average of 536 μg/day per animal. This value appears fairly high, but it must be noted that in our earlier experiments younger animals were too frequently encountered which responded to similar poisoning with a urinary total porphyrin excretion above 500 μg/day. 39.1 per cent of the urinary porphyrin was 7-COOH porphyrin and uroporphyrin. Group 2 was poisoned with HCB first for 2.5 months and then, after a 4-month interval, for a further 11 weeks. Before the start of the second poisoning period, the porphyrin excretion had still not normalised: the animals remained porphyric, with an average urinary total porphyrin excretion of 58 μg/day (±14.1 S.D.; n = 10). In week 10 of the second poisoning period, the extent of porphyrin excretion was roughly the same as for the freshly-poisoned animals: the average was 522.65 μg/day per animal, the 7-COOH porphyrin and uroporphyrin making up 35.1 per cent of this. Naturally, in both group 1 and group 2 the signs of toxic porphyria were strongly manifested: the urinary coprophyrin and the faecal proto- and coproporphyrin levels were considerably elevated. In accordance with our previous experience, in group 3 uroporphyrinuria did not develop;

Table 1. Porphyrin excretion (μg/day per animal) at week 10 of treatment, and the total liver weights in the various groups of rats

	Groups*			
	1	2	3	4
Urinary porphyrins:				
proto	10.93	0.57	0.56	
3-COOH	5.77	6.36	0.20	
copro	230.98	245.20	14.24	6.20
5-COOH	49.63	56.55	0.63	
6-COOH	28.98	30.50	0.23	
7-COOH	67.50	64.06	0.53	
uro	142.21	119.41	1.09	
Total	536.00	522.65	17.48	7.35
Faecal porphyrins:				
proto	64.89	81.94	122.30	8.70
copro	135.00	117.67	14.15	3.10
Total†	361.22	359.07	151.47	11.80
Total liver weight:				
average (g)	35.38 ($n = 7$)	38.67 ($n = 5$)	20.85 ($n = 4$)	20.01 ($n = 7$)

* Group 1 was fed for 11 weeks with feed containing 0.2% (w/w) HCB. Group 2 was made porphyric by treatment with HCB, it next received normal feed transitionally, finally, it was again given feed containing 0.2% (w/w) HCB. Group 3 received normal feed but drank water containing 10% (v/v) ethanol. Group 4 consisted of untreated animals.

† As for the urinary porphyrins, faecal total porphyrins also contain other porphyrins of different number of carboxyl groups.

Porphyrin values represent averages from 5 animals in each group.

the chronic alcohol consumption caused only a moderate degree of toxic porphyria [15].

In the case of the control animals, microscopic examination of the isolated hepatocytes revealed regular, rounded cells with a fairly homogenous size distribution. In the ethanol-treated group, there was a perceptibly larger scatter towards both larger and smaller cell diameters. A striking number of strongly enlarged cells could be seen in both groups treated with HCB. It appears that the change in size of the hepatocytes is in qualitative agreement with the total liver weights in the various groups (see Table 1).

One of the ESR spectra recorded on spin-labeled hepatocyte cell suspension is presented in Fig. 1. Three parallel spectra were recorded on the samples from each animal. Since the conditions of trypsin digestion could be ensured as strictly the same only in the event of samples prepared on the same day, we used the expression $\Delta S/S_{control}$ for the comparison of the order parameters, where ΔS is the difference between the order parameters for untreated and treated group samples prepared on one and the same day (Fig. 2). This mode of presentation demonstrates well that $\Delta S/S_{control} > 0$ for all experimental data, i.e. each of the order parameters for the treated groups was systematically smaller than that for the control sample prepared on the same day. The ratios for the different groups, involving all measurements,

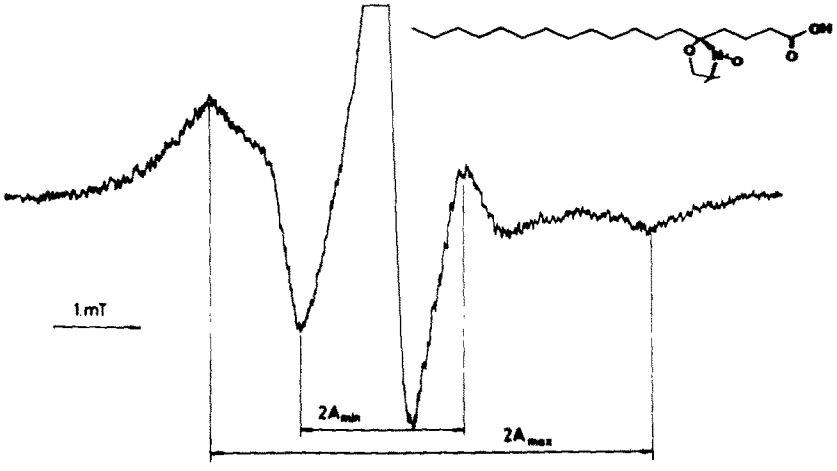

Fig. 1. The molecular structure and ESR spectrum of 5-doxylstearic acid spin probe in isolated hepatocytes of rat. Lettering is explained in Materials and Methods. ESR spectra were recorded at room temperature using the 100 kHz modulation technique.

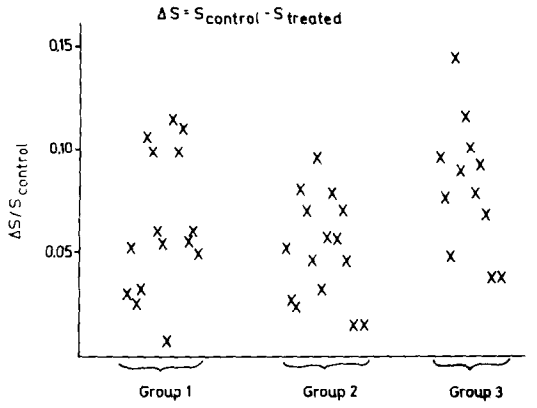

Fig. 2. Comparison of the order parameters for the different groups. ΔS means the difference between the order parameter for the untreated and treated group (samples prepared on one and the same day). Each of the order parameters for the treated groups was systematically smaller than that for the control sampled prepared on the same day.

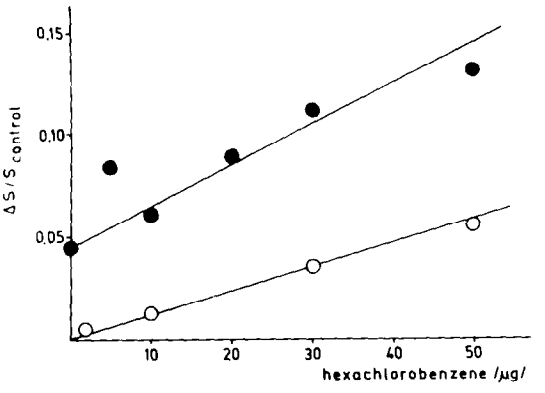

Fig. 3. Membrane-fluidizing effect of HCB. With the aim of incorporating HCB into the membrane of the control isolated hepatocytes, increasing amounts of HCB were dried onto the bottom of glass tubes, and after addition of the cell suspensions they were mixed with a vortex mixer. ○: The hepatocytes took up only 3% of the total quantities of HCB indicated along the axis. ●: In order to promote HCB absorption, the measurement series was repeated after addition of 2 μl of extra ethanol, where HCB incorporation could be increased to 4%. Each point represents the average of three measurements. HCB led to a decrease in the order parameter in both measurement series.

independently of the time of preparation, are listed in Table 2. It is clear that in the three treated groups the order parameter proved significantly smaller than in the control group. However, the order parameters for the three treated groups did not differ significantly from one another. The percentage decreases in the order parameter in the individual groups (compared to the control value) are similarly given in Table 2.

Subsequently, we attempted to incorporate HCB into hepatocytes obtained from the control animals. Naturally, in the aqueous medium the hepatocytes were able to take up only a very small proportion of the total quantity of HCB, which is virtually insoluble in water. Accordingly, in order to promote absorption, we repeated the measurement series after the addition of extra ethanol over and above that added with the spin probe and the rotenone, so that the final concentration was 5% (v/v). Measurement of the HCB remaining in the tubes showed that normally the HCB absorbed was on average only about 3 per cent of the quantity dried on the bottom of the tube, and this was increased to 4 per cent by the addition of the extra ethanol. Despite the unfavourable absorption, as can be seen in Fig. 3, the increasing quantity of HCB led to a surprising decrease in the regular order parameter in both measurement series, thereby clearly demonstrating the strong membrane-fluidizing effect of HCB. The incorporation of HCB in this way caused no microscopically visible change at all in the hepatocyte suspension.

The effect of ethanol on the control hepatocyte suspension was examined similarly. Various amounts of extra ethanol were added to samples prepared for

Table 2. Comparison of the order parameters for the different groups of rats

	Groups*			
	1	2	3	4
Order parameter				
Average	0.527	0.536	0.510	0.560
S.D.	0.029	0.026	0.031	0.027
	(n = 21)	(n = 15)	(n = 12)	(n = 21)
Order parameter (%) (compared to the control value)	94.0	95.7	91.1	100.0%
Significant difference between groups				
4 – 2	is	P = 0.032	(at 95% level)	
4 – 1	is	P = 0.003	(at 99% level)	
4 – 3	is	P = 0.001	(at 99% level)	
1 – 2	is not	P = 0.600	(at 95% level)	
2 – 3	is not	P = 0.069	(at 95% level)	
1 – 3	is not	P = 1.434	(at 95% level)	

* Group 1: treated with HCB.
Group 2: treated with HCB for a longer period.
Group 3: treated with ethanol.
Group 4: untreated control.

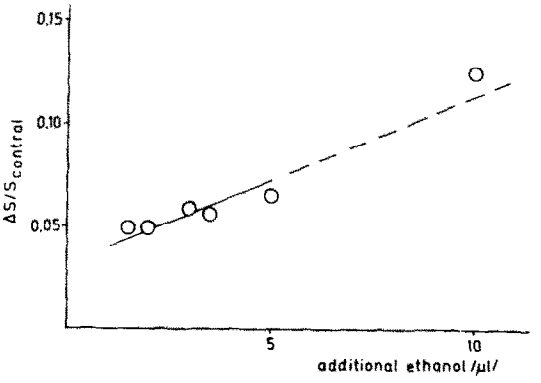

Fig. 4. Membrane-fluidizing effect of ethanol. Various amounts of extra ethanol were added to samples of control hepatocyte suspension prepared for spectral recording. Ethanol caused a decrease in order parameter which was accompanied by cell lysis, too. That is why the second half of the curve has been drawn with dashed line.

spectral recording. Incorporation from the medium into the membranes can not pose a problem for water-soluble ethanol. In accordance with this, increase of the ethanol concentration was accompanied by a strong decrease in the order parameter (Fig. 4). At the same time, the ethanol caused damage in the cells, to an extent depending on its concentration: microscopic examination revealed that when 2 μl extra ethanol was added, besides that added with the spin probe and rotenone, only about 70 per cent of the cells survived; the addition of 5 μl ethanol led to a survival rate of about 50 per cent; and 10 μl caused practically complete cell lysis, for in this case no intact cells at all could be observed by microscope.

DISCUSSION

We earlier drew attention to the fact that in the course of hepatic porphyrin synthesis first the 5-aminolaevulinic acid and then the porphyrin must penetrate the mitochondrial membrane, and thus any change in the membrane permeability may be an important factor influencing the process [10, 11, 16]. On the development of uroporphyrinuria in HCB-induced experimental porphyria, porphyrin excretion in the liver occurs not only into the bile capillaries, but also into the intercellular substance; accordingly, this indicates a pathologic permeability change in the cell membranes [10]. Certain of the steps in the porphyrin synthesis are changes catalyzed by enzymes with a lysosomal or mitochondrial localization, and in the normal state a role is played in these changes only by the penetration through the mitochondrial membrane; nevertheless, the above data show that in PCT and in experimental porphyria modelling PCT it is necessary to reckon with pathologic changes not only in the mitochondrial membrane, but in the lipoid membranes of the organelles in general too. In the present experiment, the strong trypsin digestion allowed part of the spin label to reach all membrane types in the hepatocyte thus the spectrum obtained is the average of the information from the various sites. Although this circumstance was advantageous, it must be noted, however, that studies with isolated membranes may yield different results from studies with cells.

Spin probing is a most sensitive method to measure structural changes of biological membranes in near physiological conditions. When using 5-doxyl stearic acid, a fatty acid analog, the average molecular ordering of the hydrocarbon matrix close to the interface can be quantitatively measured [13, 14]. The spectral data are quantitated by introducing the order parameter S which is a measure of the average orientation of the doxyl-carrying methylene segment with respect to the transmembrane axis. The lower the order parameter S, the less regularly the acyl chains extend towards the membrane interior.

In our experiments, the order parameter decreased significantly compared to that for the untreated control group in both the alcohol-treated group and the groups treated with HCB for various times. This finding proves that these compounds, which are able to enhance the porphyrin metabolism, display fluidizing properties in natural membranes. To a first approximation, this result is similar to the conception of Neilson *et al.* [17, 18]; in chick embryo hepatocytes, they examined the porphyrin-inducing effects of adamantane and benzene analogues [17] and alfaxolone and its derivatives [18] and, utilizing the experimental results obtained earlier with these same compounds on liposomes with the spin labeling technique [19–21], they concluded that those compounds which exhibit a porphyrin-inducing effect must be lipophilic and must posess an uncharged polar group [17], and they further assumed that there is a correlation between the membrane-fluidizing ability and the porphyrin-inducing ability [17, 18].

However, a difference must be made between the effects of the two compounds used in our experiments. On either acute [22, 23] or chronic [15] treatment, ethanol enhances the total porphyrin excretion only weakly and causes no appreciable uroporphyrinuria either, as shown by the data in Table 1; in contrast, HCB produces a considerable increase in the total porphyrin excretion, with a noteworthy uroporphyrin proportion. In the chronically poisoned groups, however, there was no significant difference between the membrane-fluidizing effects brought about by the two compounds (see Table 2). Although the membrane-fluidizing and porphyrin-inducing effects of the compounds do display similar tendencies, our experiments do not reveal a clear-cut linear quantitative correlation between them. It should be noted that, on the basis of experience acquired with alcohol and other anaesthetic compounds, Pang *et al.* [24] and Turner and Oldfield [25] recently discussed the difficulties of the quantitative application to physiological conditions of membrane-fluidizing effects observed on liposomes. Pang *et al.* similarly drew attention to the fact that the membrane composition has a considerable influence on the fluidizing abilities of the various compounds [24].

The difference between the mechanisms of action of ethanol and HCB may stem from a number of factors: (a) The lipid–buffer partition coefficient of the water-soluble ethanol in the dimyristoylphosphatidylcholine system is 0.44 [24]. This value would hardly differ appreciably from the partition coeffi-

cient found in an *in vivo* system. In contrast, HCB is practically insoluble in water, and therefore all of the HCB passing into the organism will dissolve in the lipids, including the lipoid structures. (b) The molecules differ considerably in size and structure, and in all probability they will therefore display different incorporations into the membranes. (c) The organism can extend a relatively rapid and effective defence against alcohol [26]; this is again in contrast with HCB, for the catabolism of HCB entering and being absorbed in the organism is very slow [16, 27–29], and hence this lipophilic compound with its very low reactivity is accumulated in the organism [16, 30, 31]. Our experiments suggest that, even though both ethanol and HCB are able to fluidize natural membranes, the fact that only HCB can induce uroporphyrinuria is to be attributed to the above differences in their properties.

The addition of ethanol to the control cell suspension in our experiments also changed the molarity of the medium of the system; thus, the damage to the cells need not have been a consequence merely of the fluidizing action of the ethanol, for the difference in osmotic pressure in the medium and within the cell could also have played a role. Nevertheless, as long as ordered structures were present in the system (and the majority of the cells remained intact after the addition of 1 or 2 μl ethanol), the spin-labeling technique could be employed effectively for the study of membrane fluidity. One thing should be stressed above all. A certain amount of ethanol (1.5–2 μl) decreased the original order parameter of the system to about 95 per cent, and this amount of ethanol certainly did not cause a considerable osmotic pressure difference, but there was a perceptible reduction in the survival of the cells. In contrast, in the case of HCB incorporation, the quantity of HCB necessary to decrease the order parameter to 95 per cent did not lead to a perceptible change in the survival of the cells. It may be concluded from this that the membranes are able over a long period to endure a locally fairly high HCB concentration accumulated in chronic HCB poisoning; this concentration probably exceeds the concentration of ethanol incorporated in the membrane (for only a short time because of the rapid catabolism) on acute alcohol poisoning. That the membrane does undergo lysis during chronic poisoning with either ethanol or HCB is indicated by the electron-microscopic findings [32, 33]. This membrane disorganization, however, can clearly be ascribed not only to the primary fluidizing effects of the compounds, but also to the consequences of the altered lipid metabolism striving to eliminate the damaging effect on the membrane. In our view, therefore, whereas strongly lipophilic compounds of low reactivity are able to cause uroporphyrinuria directly and by means of their secondary effects, compounds with different properties (e.g. ethanol), which likewise exert membrane-fluidizing effects, can at most only aggravate the developed pathologic state.

In our experiments, HCB significantly reduced the order parameter of isolated hepatocyte membranes. In principle, there are two means for HCB to be incorporated into the membrane: (a) The HCB is incorporated between the lipid double layer constituting the membrane. (b) The HCB molecule is incorporated between the fatty acid chains. Our experimental results support the second possibility, since the data obtained with the selected spin probe revealed a decrease of the ordering of the membrane at the level of the 5th C atom in the fatty acid chains.

When a membrane fluidity change arises on the action of some drug, the organism strives to correct this via a changed membrane lipid composition. Information regarding this is already available in connection with ethanol [34–36]. HCB incorporation obviously likewise induces a change in the lipid metabolism. At any event, this is indicated in our present experiments by the change in the liver weight and by the increased size of the hepatocytes. The difference between the order parameters for the groups undergoing shorter and longer HCB treatment (groups 1 and 2) was not significant (see Table 2). We attribute this to the fact that an approximate equilibrium probably arises between the primary fluidizing effect of the HCB and the defence by the organism (fatty degeneration of the liver) after the development of the porphyric state. The considerable concentration of accumulated HCB will then ensure that no significant change will result in the state of the membranes, either if the poisoning is discontinued, or if it is recommenced after a certain interval.

Disregarding Turkish porphyria, where the cause of the disease was proved to be HCB [37], in the case of the human disease PCT it can generally be justifiably assumed that HCB poisoning is not the cause. As the explanation of the pathomechanism in these cases, in addition to the inherited or acquired urogen-D activity decrease, only the changed membrane composition remains from the above possibilities. In our view, if for some reason (e.g. poisoning with various drugs, malnutrition, or abnormalities in absorption or utilization; or possibly a combination of these) a change occurs in the membranes of the organelles, as a consequence of which the uroporphyrin passes from the cytosol during the porphyrin metabolism, not only into the mitochondrium but, as a result of the membrane disorganization and the closely related permeability change, into the intercellular space, then the organism strives to replace the uroporphyrin excreted with the urine, i.e. the mitochondrial haem requirements will be covered by an enhanced porphyrin synthesis. Accordingly, therefore, we ascribe an important role to the membrane damage in the development of PCT.

Finally, it is noted that the membrane-damaging effect of HCB demonstrated in our present experiments is in agreement with its action on the liposomal system, where it was shown earlier to increase the permeability of the membrane with respect to water-soluble substances [10].

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REFERENCES

1. J. P. Kushner, A. J. Barbuto and G. R. Lee, *J. clin. Invest.* **58**, 1089 (1976).

2. G. H. Elder, *Semin. Hemat.* **14**, 227 (1977).
3. G. H. Elder, G. B. Lee and J. A. Tovey, *New Engl. J. Med.* **299**, 274 (1978).
4. A. V. Benedetto, J. P. Kushner and J. S. Taylor, *New Engl. J. Med.* **298**, 858 (1978).
5. H. De Verneuil, G. Aitken and Y. Nordmann, *Human Genet.* **44**, 145 (1978).
6. R. Tiepermann, G. Topi, L. D'Alessandro Gandolfo and M. Doss, *J. clin. Chem. clin. Biochem.* **16**, 52 (1978).
7. G. H. Elder, D. M. Sheppard, R. E. De Salamanca and A. Olmos, *Clin. Sci.* **58**, 477 (1980).
8. N. Simon, J. Hunyadi, A. Szörényi and A. Dobozy, *Hautarzt* **29**, 378 (1978).
9. G. H. Elder, in *Heme and Hemoproteins* (Eds F. De Matteis and W. N. Aldridge), p. 157. Springer-Verlag, Berlin (1978).
10. F. Kószó, Cs. Siklósi and N. Simon, *Biochim. biophys. Acta* **363**, 182 (1974).
11. N. Simon, Cs. Siklósi and F. Kószó, in *Porphyrins in Human Diseases* (Ed. M. Doss), p. 432. Karger, Basel (1976).
12. M. Doss, in *Progress in Thin-Layer Chromatography and Related Methods* (Eds A. Niederwieser and G. Pataki), Vol. 3, p. 147. Ann Arbor Science Publishers Inc., Ann Arbor (1972).
13. O. H. Griffith and P. C. Jost, in *Spin Labeling Theory and Applications* (Ed. L. J. Berliner), Vol. 1, p. 453. Academic Press, New York (1976).
14. R. D. Sauerheber, L. M. Gordon, R. D. Crosland and M. D. Kuwahara, *J. membr. Biol.* **31**, 131 (1977).
15. F. Kószó, Cs. Siklósi and N. Simon, *Pharmac. Biochem. Behav.* **13**, 325 (1980).
16. F. Kószó, Cs. Siklósi and N. Simon, *Biochem. biophys. Res. Commun.* **80**, 781 (1978).
17. I. R. Neilson, F. T. Chaykowski, M. A. Singer and G. S. Marks, *Biochem. Pharmac.* **28**, 3589 (1979).
18. I. R. Neilson, M. A. Singer and G. S. Marks, *Molec. Pharmac.* **18**, 144 (1980).
19. F. T. Chaykowski, J. K. S. Wan and M. A. Singer, *Chem. Phys. Lip.* **23**, 111 (1979).
20. M. A. Singer and J. Wan, *Biochem. Pharmac.* **26**, 2259 (1977).
21. D. K. Lawrence and E. W. Gill, *Molec. Pharmac.* **11**, 280 (1975).
22. K. Franke and R. Fikentscher, *Münch. med. Wschr.* **82**, 171 (1935).
23. K. E. L. Mc Coll, G. G. Thompson, M. R. Moore and A. Goldberg, *Eur. J. clin. Invest.* **10**, 107 (1980).
24. K.-Y. Y. Pang, L. M. Braswell, L. Chang, T. J. Sommer and K. W. Miller, *Molec. Pharmac.* **18**, 84 (1980).
25. G. L. Turner and E. Oldfield, *Nature, Lond.* **277**, 669 (1979).
26. C. S. Lieber, *Semin. Hemat.* **17**, 85 (1980).
27. H. M. Mehendale, M. Fields and H. B. Matthews, *J. agric. Fd Chem.* **23**, 261 (1975).
28. G. Koss, in *Porphyrins in Human Diseases* (Ed. M. Doss), p. 414. Karger, Basel (1976).
29. H. Lui, R. Sampson and G. D. Sweeney, in *Porphyrins in Human Diseases* (Ed. M. Doss), p. 405. Karger, Basel (1976).
30. J. G. Vos, H. L. Van der Maas, A. Müsch and E. Ram, *Toxic. appl. Pharmac.* **18**, 944 (1971).
31. M. D. Stonard, *Br. J. Haemat.* **27**, 617 (1974).
32. L. Biempica, N. Kosower, H. Marcus and S. Goldfisher, *Archs Path.* **98**, 336 (1974).
33. M. Kujawa and H. Karwacka, *Expl. Path.* **18**, 329 (1980).
34. D. A. Johnson, N. M. Lee, R. Cooke and H. H. Loh, *Molec. Pharmac.* **15**, 739 (1979).
35. D. A. Johnson, H. J. Friedman, R. Cooke and N. M. Lee, *Biochem. Pharmac.* **29**, 1673 (1980).
36. N. M. Lee, H. J. Friedman and H. H. Loh, *Biochem. Pharmac.* **29**, 2815 (1980).
37. C. Cam and G. Nigogosyan, *J. Am. med. Ass.* **183**, 88 (1963).