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THE EFFECTS OF CERTAIN BARBITURATES ON THE HEPATIC PORPHYRIN METABOLISM OF RATS

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Abstract—(1) Some barbiturates when given to rabbits cause a rise in urinary coproporphyrin excretion while others, suspected of provoking acute attacks of porphyria in man, do not. To investigate this anomaly, the levels of hepatic enzymes occurring early in the biosynthetic pathway have been examined in rats, treated with these barbiturates. (2) Of the nine barbiturates tested all significantly raised the hepatic level of ALA. synthetase activity in the rat. (3) These barbiturates may be classified into four significantly different groups, which correspond to the groups of the earlier classification based on urinary coproporphyrin excretion. (4) It is suggested that these results show a direct reason for contraindication of any barbiturates in porphyria. (5) The mechanism of this elevation of enzyme activity by barbiturates is discussed.

MANY barbiturates are known to adversely affect acute intermittent porphyria, either by provoking attacks or if taken after the beginning of an attack, by profoundly aggravating the severity of neurological manifestations.[1] When barbiturates were administered to rabbits, it was found that out of nine barbiturates, three of them, each possessing one or two allyl groups, caused a marked increase in urinary coproporphyrin excretion, while the remaining six caused a slight or no increase in porphyrin excretion,[2] although all of these drugs had been associated with provocation and aggravation of the human disease.

It is now known that in a number of experimental porphyrias induced in animals by drugs, and in several of the hepatic porphyrias including acute intermittent porphyria, a constant finding is an elevation of hepatic ALA synthetase* the initial and rate limiting enzyme of haem biosynthesis.[3, 4] For these reasons it seemed profitable to determine the effect of these same nine barbiturates on the hepatic ALA synthetase activity of an experimental animal, particularly noting the effect of those barbiturates which had failed to cause any increase of porphyrin excretion in the previous study.

The subsequent enzyme of the haem biosynthetic pathway, ALA dehydrase, is also known to be rate limiting in some systems of porphyrin biosynthesis[5] and exhibits many characteristics of an allosteric enzyme suggestive of a regulatory role.[6] ALA dehydrase activity was therefore also measured in these experiments.

MATERIALS AND METHODS

The experimental animals. Male Sprague–Dawley rats weighing about 200 g at the

* Abbreviation: ALA δ aminolaevulinic acid.

commencement of the experiment were used. These were used in groups of twelve, six test and six control animals, caged in pairs and fed on standard rat diet 41. Drugs were administered intraperitoneally as an aqueous solution or orally as a propylene glycol syrup. The livers were excised under ether anaesthetic, immediately chilled in ice-cold saline, weighed, and aliquots removed for enzyme and porphyrin assays. The animals were killed by exsanguination before they regained consciousness. Liver weights are expressed as g wet weight.

The barbiturates. Table 1 lists those used and the routes of administration. Hereafter

TABLE 1. BARBITURATES AND ANIMAL EXPERIMENTAL PLAN

Approved name	Chemical name	No. of animals	Method of dosage
Dial	Diallyl barbituric acid	5	Oral
Allobarbitone	5 Allyl 5 isopropyl barbituric acid	5	Oral
Quinalbarbitone	5 Allyl 5·1 methyl butyl barbituric acid	5	Oral
Barbitone	5·5 diethyl barbituric acid	5	i.p.
Pentobarbitone	5 ethyl 5·1 methyl butyl barbituric acid	5	i.p.
Phenobarbitone	5 ethyl 5 phenyl barbituric acid	6	i.p.
Butobarbitone	5 butyl 5 ethyl barbituric acid	6	Oral
Thiopentone	5 ethyl 5·1 methyl butyl thio barbituric acid	5	i.p.
Amylobarbitone	5 ethyl 5 isopentyl barbituric acid	6	i.p.

these barbiturates will be named by their approved name. The dose administered was the anaesthetic dose as recommended by Barnes and Eltherington.[7] They were administered in the morning to allow minimal disturbance of the animals nocturnal feeding habits.

Enzyme assays. ALA synthetase was assayed by a micro-modification of the method of Marver *et al.*[8] as described by Dowdle *et al.*[9] on a 1 in 4 liver homogenate. ALA dehydrase was assayed as for blood on a 1 in 10 liver homogenate in 0·15 M KCl, (Lichtman and Feldman.[10])

Porphyrins. These were measured by the method of Rimington.[11]

Statistics. These were carried out using Students *t*-test, and all results were expressed as the mean $\pm$ standard deviation except where otherwise indicated.

RESULTS

The time course of barbiturate stimulation of ALA synthetase activity was studied in detail with phenobarbitone which was administered (at anaesthetic dosage) every 24 hr for 12 days. The activity of hepatic ALA synthetase rose rapidly to a peak at about 70 hr plateauing about 200 hr (Fig. 1). From these results it was decided to administer the drugs for 6 days. The levels of ALA synthetase activity following phenobarbitone administration by the two different methods of drug administration were not significantly different (Table 2). The parameters measured on each liver were ALA synthetase, ALA dehydrase, coproporphyrin and protoporphyrin (Table 3). Also measured were body weight and liver weight. There was no significant difference in body weight change or liver weight per 100 g body weight in any of the groups.

The following were significantly elevated; hepatic ALA synthetase activity in all samples, with levels of significance, for thiopentone ($P < 0·02$), for amylobarbitone

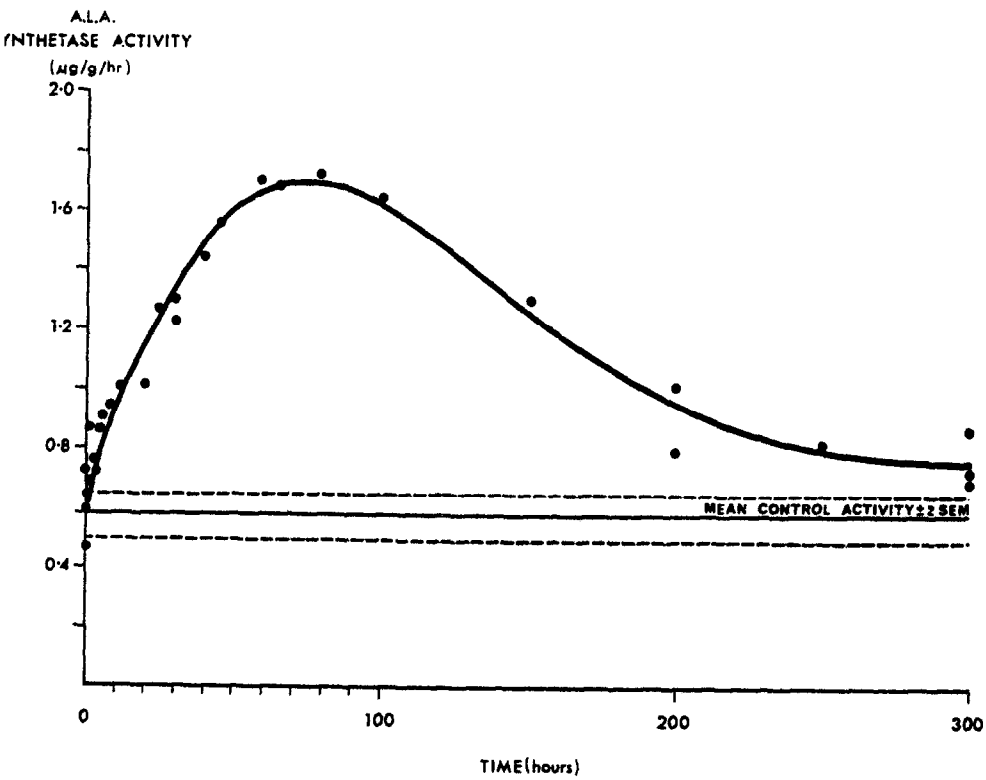

FIG. 1. Time course; Effect of phenobarbitone on ALA synthetase activity. Each point represents one rat. Phenobarbitone was administered at anaesthetic dosage every 24 hr during the period of the experiment.

TABLE 2. EFFECT OF DIFFERENT MEANS OF DOSAGE

Barbiturate	No. of rats used	ALA synthetase activity (μg ALA/g wet wt. liver/hr)
Phenobarbitone (intraperitoneally)	6	1·242 ± 0·227
Phenobarbitone (orally)	5	1·201 ± 0·228

($P < 0.01$) and for the rest ($P < 0.001$); hepatic ALA dehydrase activity in Dial with a level of significance ($P < 0.05$); hepatic coproporphyrin in Dial ($P < 0.001$) and in allobarbitone and quinalbarbitone ($P < 0.01$). No other parameters were significantly elevated. On the basis of the results these barbiturates may be separated into four significantly different groups (Fig. 2). In the first group (Dial) the hepatic ALA synthetase activity was significantly greater than the activity in the second group (allobarbitone and quinalbarbitone) ($P < 0.001$). The activity in the second group was greater than the activity in the third group (phenobarbitone, barbitone and

TABLE 3. THE EFFECT OF CERTAIN BARBITURATES ON BODY AND LIVER WEIGHTS, PORPHYRINOGENIC ENZYMES AND PORPHYRINS

Drug	No. of rats	Increase in body wt. (g)	Wt. liver/ 100 g body wt. (g)*	ALA synthetase activity (μg/g/hr)*	ALA dehydrase activity (mg/g/hr)*	Copropor- phyrin (μg/g)*	Protopor- phyrin (μg/g)*
Dial	5	55·0 ± 7·1	4·12 ± 0·50	1·916 ± 0·250	13·48 ± 1·19	7·50 ± 0·700	8·00 ± 0·100
Allobarbitone	5	55·0 ± 12·1	4·76 ± 0·40	1·452 ± 0·124	12·266 ± 0·61	0·91 ± 0·090	0·89 ± 0·410
Quinalbarbitone	5	58·0 ± 20·8	3·99 ± 0·58	1·440 ± 0·112	11·89 ± 1·56	1·03 ± 0·610	0·83 ± 0·350
Barbitone	5	54·0 ± 8·2	4·52 ± 0·41	1·253 ± 0·196	11·97 ± 1·24	0·152 ± 0·112	0·137 ± 0·101
Pentobarbitone	5	64·2 ± 12·0	4·10 ± 0·22	1·252 ± 0·272	12·04 ± 2·33	0·171 ± 0·156	0·149 ± 0·150
Phenobarbitone	6	47·0 ± 15·0	4·75 ± 0·39	1·242 ± 0·227	12·93 ± 0·89	0·074 ± 0·010	0·056 ± 0·014
Butobarbitone	6	58·0 ± 10·1	3·67 ± 0·42	0·985 ± 0·84	11·17 ± 0·84	0·110 ± 0·030	0·090 ± 0·050
Thiopentone	5	65·0 ± 12·7	4·26 ± 0·52	1·014 ± 0·253	11·5 ± 0·67	0·040 ± 0·020	0·076 ± 0·010
Amylobarbitone	6	60·0 ± 54·0	4·21 ± 0·43	1·056 ± 0·186	10·65 ± 1·53	0·038 ± 0·024	0·043 ± 0·030
Control	25	64·6 ± 10·1	4·26 ± 0·93	0·576 ± 0·208	11·34 ± 1·69	0·111 ± 0·050	0·088 ± 0·750

* In all cases g/wet weight liver.

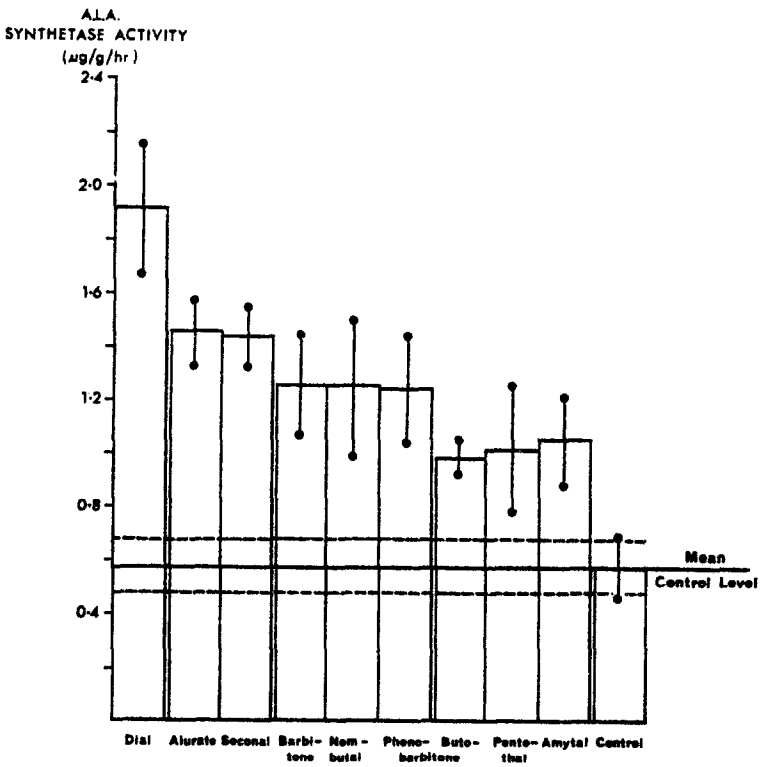

FIG. 2. The effects of certain barbiturates on hepatic ALA synthetase activity in the rat. Each block of the histogram represents the mean activity. Vertical bars show the standard deviation.

pentobarbitone) ($P < 0{\cdot}05$) and the activity in this third group was greater than in the fourth group (thiopentone, amylobarbitone and butobarbitone) ($P < 0{\cdot}001$) which was greater than the activity in the control group ($P < 0{\cdot}001$).

DISCUSSION

It has been shown that of nine barbiturates tested, all significantly elevated hepatic ALA synthetase activity in the rat. Hepatic porphyrins increased with three of these barbiturates, Dial, allobarbitone and quinalbarbitone, that is, the three which previously caused the greatest excretion of urinary porphyrins in rabbits. Dial also elevated ALA dehydrase activity.

On the basis of the elevations of ALA synthetase activity, these barbiturates may be classified into four groups.

(1) Dial, causing a large rise of hepatic ALA synthetase activity, ALA dehydrase activity and of hepatic copro- and protoporphyrin.

(2) Allobarbitone and quinalbarbitone, which caused a lesser rise of hepatic ALA synthetase activity and a small rise of hepatic copro- and protoporphyrins.

(3) Barbitone, pentobarbitone and phenobarbitone which caused a rise of hepatic ALA synthetase activity.

(4) Thiopentone, amylobarbitone and butobarbitone which caused a smaller but significant rise of hepatic ALA synthetase activity.

This classification is the same as that previously based on urinary coproporphyrin excretion.[2] The main difference is that in group 4 there was no elevation of urinary coproporphyrin excretion in the previous study, whilst in the present study, the level of ALA synthetase was significantly elevated in all groups including group 4. This underlines the rule that all barbiturates are contraindicated in patients with hepatic porphyrias since in them there is already an elevation of hepatic ALA synthetase.

Hepatic ALA dehydrase was also raised in the case of Dial. The relative quantities of ALA synthetase and ALA dehydrase are such that a large rise in ALA synthetase activity does not necessitate a parallel rise in the activity of ALA dehydrase, to cope with the increased production of ALA, and it is, therefore, unlikely that control of ALA dehydrase activity constitutes a method of control in the liver. It is consequently not surprising that the levels of ALA dehydrase have not been raised.

The hepatic levels of copro- and protoporphyrin were only raised by the drugs containing an allyl group, as was also found by Goldberg.[2] When the level of porphyrin formation is higher there is clearly some accumulation of porphyrins leading to these higher hepatic levels.

The biochemical relationship between acute porphyria and its sensitivity to barbiturates has in the past been ascribed to blocks in the pathway of purine biosynthesis,[12] or to impairment of acetycholine synthesis by restriction of acetyl CoA through a deficiency of ATP limiting its synthesis.[13] The latter hypothesis was strengthened by the work of Kyogoku *et al.*[14] who showed that barbiturates form highly specific hydrogen bonded adenine derivatives, and suggested that this strong complex is an explanation of the physiological activity of barbiturates.

It is now known that in hepatic porphyrias there is an over-production of the initial and rate-limiting enzyme of porphyrin biosynthesis, ALA synthetase (succinyl CoA: glycine succinyl transferase). This was first demonstrated by Granick and Urata[3] who found that liver mitochondria from guinea pigs, made porphyric with 3·5 dicarbethoxy 1·4 dihydrocollidine, synthesised very much greater quantities of δ-aminolaevulinic acid from citric acid cycle intermediates and particularly, succinyl CoA. Their results indicated that the main reason for increased synthesis of δ-aminolaevulinic acid was not increased succinyl CoA but an increase in the enzyme ALA synthetase. It has further been shown that the biochemical disturbance of human porphyria is associated with the over-production of hepatic ALA synthetase.[4, 9, 15] Thus the possible mechanisms of barbiturate action in haem biosynthesis are three in number:

(1) *By control of production of succinyl CoA.* The importance of this mechanism is not clear; it might, however, have some part in control of haem biosynthesis since the experiments of Ludwig *et al.*[16] have shown that malonate, an inhibitor of succinate dehydrogenase, increases porphyrin production 10-fold in the rat.

(2) *End product inhibition* of enzyme activity is a much more clearly established method of control in this system. This method works by change of enzyme activity directly, possibly by alteration of the enzyme's spatial conformation.

(3) *End product repression*, which involves repression of the de novo synthesis of ALA synthetase. This is more indirect in its action involving the apparatus for protein synthesis.

Barbiturates could involve any of these three mechanisms in order to increase the activity of hepatic ALA synthetase. Phenobarbitone induces an increase in activity very rapidly within 1 hr of starting drug administration; the increase becomes statistic-

ally significant at about 2 hr after initiation. This means that the method of action must be a rapid one. Succinyl CoA regulation and end product inhibition are both relatively rapid processes. Although Granick[19] and Waxman *et al.*[2] have found evidence for end product repression in the liver, neither have found any evidence for end product inhibition in mammalian or avian systems, the only evidence for this being bacterial.[17] It would therefore seem possible that the method of barbiturate initiation of porphyrin biosynthesis lies in the region of succinyl CoA formation.

On the other hand, the method of control of this pathway both normally and drug treated systems is thought to be by de novo induction of enzyme synthesis, as suggested by work using nucleic acid and protein synthesis inhibitors. In addition, phenobarbitone has been shown to increase the microsomal mixed function oxidase, cytochrome P.450[21], a haemoprotein central in hepatic oxidative transformation. As a haemoprotein its increased synthesis probably relies on the increased function of the haem biosynthetic pathway. Whether this occurs initially, due to an increase in ALA synthetase activity by methods such as citric acid cycle control or due to a decrease in the repressor haem which is being drawn off to produce cytochrome P.450 required to oxidise the barbiturate, remains to be elucidated. Thus, although rigorous proof of de novo induction is not yet available and evidence does suggest that it is the main means of regulation, rather than citric acid cycle control, it is possible that barbiturate stimulation of this biosynthetic pathway may be through either of these control mechanisms.

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