work and it is recommended for all applications where continuous changes of optical activity are involved.

SUMMARY

1. No evidence was found that heavy metal ions (Cu^{2+} , Zn^{2+} , Ni^{2+} and Hg^{2+}) catalyse the mutarotation of glucose.

2. The strong catalytic action of mutarotase was verified. That pepsin largely destroys this activity may be taken as additional proof of the protein nature of mutarotase.

3. α -Amylase was found to have no mutarotase activity.

4. The specificity of mutarotase was investigated in greater detail. It appears that the enzyme catalyses only some $\alpha \rightleftharpoons \beta$ transformations. Of the aldoses tested, glucose and galactose seem to be the best substrates, indicating that the configuration at the fourth carbon atom is not decisive. However, the mutarotation of the disaccharides lactose and maltose is accelerated to a lesser degree. The lack of a hydroxymethyl group on the fifth carbon atom (xylose) also reduces the activity. The configuration at the second carbon atom of the sugar molecule is decisive, since neither mannose nor glucosamine serves as a substrate. These studies indicate the range of specificity of mutarotase.

5. An incidental outcome of this work was that it demonstrated the general utility of automatic-recording polarimetry or rotography.

REFERENCES

- Bentley, R. & Neuberger, A. (1949). *Biochem. J.* **45**, 584.
 Blom, J. & Schwarz, B. (1952). *Acta chem. scand.* **5**, 697.
 Cecil, R. & Ogston, A. G. (1948). *Biochem. J.* **42**, 229.
 Coulthard, C. E., Michaelis, R., Short, W. F., Sykes, G., Skrimshire, G. E. H., Standfast, A. F. B., Birkinshaw, J. H. & Raistrick, H. (1945). *Biochem. J.* **39**, 24.
 Hopkins, R. H. (1953). *Nature, Lond.*, **171**, 429.
 Hudson, C. S. & Dale, J. K. (1917). *J. Amer. chem. Soc.* **39**, 320.
 Isbell, H. S. & Pigman, W. W. (1937). *J. Res. nat. Bur. Stand.* **18**, 141.
 Isbell, H. S. & Pigman, W. W. (1938). *J. Res. nat. Bur. Stand.* **20**, 773.
 Keilin, D. & Hartree, E. F. (1948a). *Biochem. J.* **42**, 221.
 Keilin, D. & Hartree, E. F. (1948b). *Biochem. J.* **42**, 230.
 Keilin, D. & Hartree, E. F. (1952a). *Biochem. J.* **50**, 331.
 Keilin, D. & Hartree, E. F. (1952b). *Biochem. J.* **50**, 341.
 Levy, G. B. (1950). *Nature, Lond.*, **166**, 740.
 Levy, G. B. (1951). *Analyt. Chem.* **23**, 1089.
 Levy, G. B., Schwed, P. & Fergus, D. (1950). *Rev. sci. Instrum.* **21**, 693.
 Theorell, H. (1951). *The Enzymes*, **2**, p. 348, ed. by J. B. Sumner and K. Myrbäck. New York: Academic Press, Inc.

The Effect of Certain Barbiturates on the Porphyrin Metabolism of Rabbits

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Since the introduction of the barbiturates to clinical medicine in 1903, there have been conflicting reports on their possible relation to human acute porphyria. Dobrschansky (1906) described a typical case of acute porphyria, without paralysis, occurring in a patient after prolonged administration of diethylbarbituric acid. Haxthausen (1927) reported the development of skin photosensitivity and the presence of excessive amounts of porphyrins in the urine of an epileptic who had been taking ethylphenylbarbiturate for some time. Eliaser & Kondo (1942), Denny-Brown & Sciarra (1945), Prunty (1946) and Jørgensen & With (1947) have described deterioration in the clinical state of acute porphyria patients with the development of severe or even fatal paralysis, after the administration of certain barbiturates, among which were

ethylphenylbarbituric acid and ethyl(1-methylbutyl)barbituric acid. Waldenström (1939, 1940) was convinced that barbiturates may precipitate attacks in cases of latent porphyria and that they seriously affect the prognosis of the disease. On the other hand, Günther (1922), Turner (1938) and Discombe & D'Silva (1945) failed to note any relation between barbiturates and acute porphyria.

Information on the relation of barbiturates to normal human porphyrin metabolism has been obtained from observations on the urinary porphyrin excretion in cases of barbiturate poisoning. Rosendorff (1910), Sowden (1910) and MacLean (1912) found no abnormal porphyrinuria in patients who had ingested single large doses of diethylbarbituric acid, while Rommel (1912) observed dark-red urine in a patient who had taken 25 g. of

the same drug. Lehmann & Zinn (1910) have described a urinary pigmentation, suggestive of porphyrin and porphobilin, in a patient who had received 0.5 g. diethylbarbituric acid. They gave this drug in prolonged dosage to one rabbit without the production of similar pigments. Animal experiments were also carried out by Laubender & Monden (1938), who investigated the effects of diethyl, bromallyl-isopropyl and ethylcyclohexenyl-barbituric acids on the urinary porphyrins of rabbits. They failed to find any such effect in all these experiments with the exception of one animal treated with diethylbarbituric acid, which showed a slight rise of urinary porphyrins.

The barbiturates comprise a group of chemically related drugs, while acute porphyria is a rare disease. Moreover, it is usually impossible to tell from published records whether the barbiturates had precipitated the overt disease in a case of latent porphyria or had in fact provoked acute

porphyria in a formerly healthy subject. To overcome this difficulty and the uncertainty in assessment of these clinical impressions, the effects of different barbiturates on the porphyrin metabolism of rabbits were studied. Out of nine barbiturates so tested, six had a significant effect on the urinary porphyrin excretion; those containing one or more allyl groups were particularly effective.

METHODS

Seventeen rabbits (average wt. 2.8 kg.) were housed in individual metabolism cages allowing the separation of urine and faeces. A preliminary base-line period of 4-7 days before drug administration was allowed during which the total daily urinary coproporphyrin and sometimes also faecal porphyrins were determined. If the animal died during an experiment, tissue porphyrins and porphobilinogen were also determined. The average duration of each course of barbiturate was 12 days. The types of barbiturates and their route of administration are recorded in Table 1.

Table 1. *Effect of barbiturates on the urinary excretion of coproporphyrin in rabbits*

The sodium barbiturates were given in equally divided dosage twice daily. A letter after a rabbit number signifies that the rabbit obtained the corresponding barbiturate on a primary course *a*, or on subsequent courses *b*, *c*, etc. Doses were administered by intramuscular injection unless indicated otherwise.

Drug	Rabbit no.	Dosage		Mean urinary coproporphyrin	
		Average level (mg./kg./day)	Duration (days)	Pre-dosage level (μg./day)	Increase (μg./day)
5:5-Diallylbarbituric acid	2 <i>a</i>	113*	15	19.7	+193.7
	1 <i>b</i>	103*	35	7.5	+169.5
	3	100	17	5.2	+119.2
	4 <i>a</i>	128*	12	8.7	+88.3
	5	101.6	7	8	+86
	6	125	9	5.3	+84
	7	114	7	2	+51
	8	148	7	3.3	+47
Sodium 5-allyl-5-(1-methylbutyl)-barbiturate	9	172	7	3.7	+42.6
	10	115	12	3.8	+22.9
	11 <i>b</i>	100	12	9.8	+20.2
	2 <i>d</i>	82.8	12	19.7	+17.3
5-Allyl-5-isopropylbarbituric acid	2 <i>b</i>	67.8†	12	19.7	+81.6
	12	76†	13	7	+40
	13	74†	12	7	+27
Sodium 5:5-diethylbarbiturate	14 <i>b</i>	171	13	5	+9.7
	13 <i>d</i>	140	12	7	+9.6
	15	169	13	1.8	+8.7
	16	144	12	2.4	+5.6
Sodium 5-ethyl-5-(1-methylbutyl)-barbiturate	11 <i>a</i>	98.5	12	9.8	+15.2
	2 <i>c</i>	110.5	12	19.7	+7.3
	4 <i>b</i>	128	14	8.7	+2.3
Sodium 5-ethyl-5-phenylbarbiturate	14 <i>a</i>	71	9	5	+14.7
	1 <i>a</i>	67	20	7.5	+13.5
	13 <i>c</i>	81	12	7	+11.7
	16 <i>b</i>	82	12	2.4	+6.8
Sodium 5-isoamyl-5-ethylbarbiturate	13 <i>b</i>	90	12	7	-2
	2 <i>e</i>	83	12	19.7	-5.2
Sodium 5-butyl-5-ethylbarbiturate	17 <i>a</i>	109.5	12	11.5	+5.5
	16 <i>a</i>	98	12	2.4	+2.3
Sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate	17 <i>b</i>	218	12	11.5	+5.2
	2 <i>f</i>	164	12	19.7	-1.7

* Some doses given by gastric intubation.

† Given by gastric intubation.

If the rabbit survived this course, analyses were continued during a recovery period of 4–7 days. After such an interval, some rabbits were started on a different barbiturate as indicated in Table 1. The maximum sub-lethal dose of each drug was employed.

Quantitative analyses

Urinary porphyrins. These were determined by the method of Rimington & Sveinsson (1950) with the following modifications. Ether-soluble porphyrins were extracted by ether:acetic acid (10:1 by vol.) and after the ether had been washed three times with water, were transferred directly into 1.4N-HCl for spectrophotometric determination (Beckman, model DU). One part of urine containing porphobilinogen, was diluted with 4 parts 2N sodium acetate buffer pH 4.22, and placed in boiling water for 20 min. Appropriate dilution for spectrophotometric determination of the total uroporphyrin was made with HCl so that the final concentration of acid was 0.5N.

Faecal coproporphyrin and tissue copro- and protoporphyrin. Faeces and tissues were ground thoroughly in a mortar. Liver tissue was in addition comminuted in a Waring Blendor. The ether-soluble porphyrins from weighed portions of faeces and tissue were extracted by repeated shaking with ether:acetic acid (10:1, v/v) until the supernatant after centrifuging showed no porphyrin fluorescence. The porphyrins were then analysed as in the case of urine.

Porphobilinogen. Porphobilinogen in urine was determined by the method of Vahlquist (1939). Tissue dispersions were treated with about an equal volume of 20% (w/v) trichloroacetic acid, the mixture was centrifuged and the supernatant analysed as in the case of urine.

Blood examination. Haemoglobin was determined by the method of Rimington (1942). Erythrocyte counts and reticulocyte counts were carried out using standard techniques.

Urinary amino acids. These were examined by methods of Datta, Dent & Harris (1950).

Identification of porphyrins

The urines from rabbits treated with sodium allyl(1-methylbutyl)barbiturate or allylisopropylbarbituric acid contained increased amounts of porphyrins, all of which were ether soluble. These urines were preserved with toluene and stored at 3°. The porphyrins were extracted with ether:acetic acid and, after the ether had been washed 3 times with water, were transferred into a minimal volume of 2.8N-HCl and then precipitated at the isoelectric point (pH 3.1). The precipitate was centrifuged, dried, and esterified by contact with methanolic HCl for 24 hr. The porphyrin esters were transferred into CHCl_3 by the addition of saturated sodium acetate, the CHCl_3 layer washed once with water, once with dilute ammonia, and twice with water, in that order. The α band (absorption band at longest wavelength) of the porphyrin ester at this stage was noted on the Hartridge Reversion Spectroscope. A portion of the ester was dried, hydrolysed with 7N-HCl for 36 hr. and chromatographed on paper (Nicholas & Rimington, 1951). The remainder was crystallized from CHCl_3 :methanol (1:5, v/v). Melting points, which were not corrected, were determined on an electrically heated micro apparatus (A. Gallenkamp and Co. Ltd.). Isomer analysis was done by the method of Chu, Green & Chu (1951).

Urines from some rabbits treated with diallylbarbituric acid contained uroporphyrin and porphobilinogen as well as an increase in ether-soluble porphyrins. These combined urines were brought to pH 3–4 with glacial acetic acid and sufficient talc was added to adsorb all porphyrins. The talc was filtered, dried, and repeatedly eluted with 2N ammonia until no porphyrin fluorescence appeared in the supernatant. The total alkaline eluates were then brought to the isoelectric point and the porphyrins thus precipitated were dried, esterified and taken into CHCl_3 as described above. The porphyrin esters were fractionated by chromatography on columns of aluminum oxide Grade IV and magnesium oxide Grade III (Nicholas, 1951). The fractions were defined by α band estimations and, after hydrolysis, by paper chromatography (Nicholas & Rimington, 1951). Isomer analysis of octacarboxylic porphyrin esters was performed by the method of Falk & Benson (1953), while that on tetra- and penta-carboxylic porphyrins was done by the method of Chu *et al.* (1951). The uroporphyrin ester was crystallized from chloroform:methanol.

RESULTS

Those barbiturates which produced the greatest increase of urinary porphyrins also caused the most prolonged hypnosis. Diallylbarbituric acid was the most effective drug, while allylisopropylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate, caused a higher excretion of urinary coproporphyrin and a more prolonged hypnosis than the remaining barbiturates. In each of three rabbits treated with diallylbarbituric acid, the drug was given sometimes by gastric intubation and sometimes by intramuscular injection. No significant quantitative difference of urinary porphyrin excretion was obtained by thus changing the route.

Urinary coproporphyrin. Table 1 records the average daily excretion of coproporphyrin in the urine during the pre-dosage period and the average deviation from this figure during administration of the drug. Coefficients of variation are not included since in almost every instance the results were quite obviously significant. Where an effective barbiturate was used, the urine coproporphyrin excretion showed a prompt increase after the 1st or 2nd day, rose to a maximum level on about the 6–7th day, remained at about that level during the subsequent administration of the drug and fell to normal within 2–3 days of its cessation.

Urinary uroporphyrin and porphobilinogen. In three rabbits (1b, 2a and 3) treated with diallylbarbituric acid, uroporphyrin and porphobilinogen appeared in the urine on the 7th, 7th and 8th days, respectively, and generally continued to be excreted for as long as the drug was given (Table 2 and Fig. 1).

Faecal coproporphyrin. In rabbit 1b the faecal coproporphyrin was determined before and during administration of diallylbarbituric acid. This showed that the faecal coproporphyrin rose at the same rate as the urine coproporphyrin from a

previous normal daily average of 38 $\mu\text{g.}/\text{day}$ to a daily average of 1495 $\mu\text{g.}/\text{day}$.

Tissue porphyrins. Table 3 summarizes the tissue analyses for porphyrin of four normal rabbits, and five rabbits which had died from barbiturate intoxication at a stage when they were excreting increased urinary coproporphyrin. There was an increase of copro- and proto-porphyrin concentrations in the bile and liver in intoxicated rabbits, but the levels in the bone marrow showed no abnormality. The liver of rabbit 10 gave a faint but definitely positive reaction for porphobilinogen. Uroporphyrin was never found in any tissue.

Blood examination. Haemoglobin determinations, erythrocyte counts and reticulocyte counts were made immediately before, during and after administration of the drug to three rabbits treated with diallylbarbituric acid and one treated with allylisopropyl barbituric acid. No significant change was observed in any of the erythrocyte counts or reticulocyte counts. In two of these rabbits (2b and 3), a slight fall (about 2 g. %) in the haemoglobin level was observed, while two rabbits (1b and 7) showed no significant change.

Table 2. *Daily urinary uroporphyrin and porphobilinogen excretion in rabbits receiving diallylbarbituric acid*

Rabbit no.	Total uroporphyrin ($\mu\text{g.}/\text{day}$)		Porphobilinogen (units*/day)	
	Average	Maximum	Average	Maximum
1b	1720	4750	456	1607
2a	360	800	78	161
3	250	340	78	109

* Vahlquist (1939).

Fig. 1. Effects of diallylbarbituric acid on the urinary uroporphyrin, coproporphyrin and porphobilinogen and faecal coproporphyrin excretion of rabbit 1b.

Table 3. *Tissue porphyrin determinations on four normal rabbits and five rabbits treated with barbiturates*

The five rabbits died while excreting excessive urinary coproporphyrin. Figures represent $\mu\text{g.}/\text{ml.}$ (bile) or $\mu\text{g.}/\text{g.}$ (liver and marrow).

Rabbit no.	Bile				Liver				Bone marrow			
	Copro-porphyrin	Proto-porphyrin	Porphobilinogen	Uro-porphyrin	Copro-porphyrin	Proto-porphyrin	Porphobilinogen	Uro-porphyrin	Copro-porphyrin	Proto-porphyrin	Porphobilinogen	Uro-porphyrin
Normal												
Control I	0.15	Nil	0.10	Nil	0.10	0.10	Nil	Nil	0.10	Nil	Nil	Nil
Control II	0.38	0.34	0.12	Nil	0.12	0.12	Nil	Nil	0.20	0.15	Nil	Nil
Control III	0.74	0.41	0.51	Nil	0.18	0.51	Nil	Nil	0.22	0.24	Nil	Nil
Control IV	Insufficient obtained	Insufficient obtained	0.23	Nil	0.02	0.23	Nil	Nil	0.18	0.16	Nil	Nil
Diallylbarbituric acid												
8	Insufficient obtained	Insufficient obtained	5.5	Nil	49	49	Nil	Nil	0.10	0.12	Nil	Nil
7	25	172	11.8	Nil	30.9	30.9	Nil	Nil	0.12	0.14	Nil	Nil
5	5.2	84.0	3.2	Nil	4.66	4.66	Nil	Nil	0.15	0.22	Nil	Nil
Sodium allyl(1-methylbutyl)barbiturate												
10	0.78	12.68	17	Nil	3.84	3.84	Faint +	Nil	0.10	0.24	Nil	Nil
9	Insufficient obtained	Insufficient obtained	1.91	Nil	1.91	1.91	Nil	Nil	0.30	0.31	Nil	Nil

Table 4. *Identification of urinary porphyrins. Summary of results*

	Free porphyrin	Porphyrin ester			M.p. of crystals (°)	Summary
		Paper chromatography (Nicholas & Rimington, 1951)	α Band	Paper chromatography (a) Chu <i>et al.</i> 1951	(b) Falk & Benson, 1953	
Diallylbarbituric acid	8 COOH	625-1	—	Mainly series III	274	Uroporphyrin III
	6 COOH	623-6	—	—	—	Hexacarboxylic porphyrin
	5 COOH	622-5	Series III	—	—	Pentacarboxylic porphyrin III
	4 COOH	621-1	Series III	—	—	Coproporphyrin III
Sodium allyl(1-methyl-butyl)barbiturate	4 COOH	621-3	Mainly series III	—	154, remelt. 172-4	Coproporphyrin III
Allylisopropylbarbituric acid	4 COOH	621-7	Mainly series III	—	150, remelt. 160	Coproporphyrin III

Urinary amino acids. In seven rabbits, in which a rise of urinary coproporphyrin was obtained, the urinary amino acid pattern was no different before and at the end of barbiturate administration.

Identification of porphyrins. The ether-soluble porphyrins obtained from the urines of rabbits treated with allylisopropylbarbituric acid or sodium allyl(1-methylbutyl)barbiturate consisted entirely of coproporphyrin III. The ester m.p.'s and the results of paper chromatograms are shown in Table 4.

The porphyrin esters obtained from the urine of rabbits treated with diallylbarbituric acid were fractionated on a magnesium oxide column. This separated first an octacarboxylic porphyrin with an α band 625.0 $m\mu$.; then two intermediate fractions with α bands 623.0 and 621.6 $m\mu$., respectively; and finally a tetracarboxylic porphyrin with α band 621.1 $m\mu$. The first fraction was rechromatographed on a magnesium oxide column and yielded a single porphyrin zone. The appearance of the crystals obtained from this zone was suggestive of uroporphyrin III. The m.p. (274°) and paper chromatography (Falk & Benson, 1953) showed the series III isomer to be that mainly present (see Table 4). The two intermediate fractions were further chromatographed on an aluminium oxide column. This allowed the separation of a main portion with α band 622.5 $m\mu$. and a lesser portion with α band 623.5 $m\mu$., which behaved as pentacarboxylic and hexacarboxylic porphyrins, respectively, when examined by paper chromatography (Nicholas & Rimington, 1951). On a paper chromatogram (Chu *et al.* 1951), the pentacarboxylic porphyrin had about the same mobility as coproporphyrin III. The final fraction was identified as coproporphyrin III by melting point and paper chromatography (see Table 4).

DISCUSSION

This work has shown that of the nine different barbiturates examined, six were capable of producing some effect on the porphyrin metabolism of rabbits. Of seventeen rabbits used, eight were given more than one barbiturate in separate courses. This was considered as a possible factor influencing results. However, another barbiturate was never started until the urinary coproporphyrin level had returned to its normal from the previous course, and the results in Table 1 show that the same drug, used on different animals, whether it was their first or subsequent barbiturate, generally gave corresponding results in porphyrin excretion. For the more effective barbiturates, sufficient numbers of rabbits were used, on a primary or subsequent barbiturate course, to demonstrate the unimportance of previous intoxication. Difference in effect upon porphyrin excretion must depend, among other things, upon the chemical constitution of the drug, the daily dosage employed and its duration, and also, possibly, upon the normal level of porphyrin excretion of the individual animal.

The results in Table 1 show that the barbiturates used may be classified in four groups according to their effectiveness (Fig. 2).

(1) Diallylbarbituric acid, which caused a considerable rise of urinary coproporphyrin in each of eight rabbits and, in three of these, the excretion also of uroporphyrin and porphobilinogen.

(2) Allylisopropylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate, which caused a moderate rise of urinary coproporphyrin.

(3) Sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)barbiturate, and sodium ethylphenylbarbiturate, which caused only a slight increase of coproporphyrin in the urine.

Fig. 2. Each vertical segment represents the average daily coproporphyrin output in a single rabbit during barbiturate administration in excess of (or less than) the normal average daily coproporphyrin excretion. I, diallylbarbituric acid; II, allylisopropylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate. III, sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)barbiturate, and sodium ethylphenylbarbiturate. IV, sodium ethyl(1-methylbutyl)thiobarbiturate, sodium isoamylethylbarbiturate, and sodium butylethylbarbiturate.

(4) Sodium ethyl(1-methylbutyl)thiobarbiturate, sodium isoamylethylbarbiturate, and sodium butylethylbarbiturate, which did not alter significantly the urinary coproporphyrin excretion.

The possession of the allyl group thus appeared to make the barbiturate more effective in producing a rise of porphyrin in the urine. These drugs also caused deeper and more prolonged hypnosis in rabbits. Drugs possessing an allyl group have been known to give rise to pathological and metabolic changes in humans and in experimental animals. Popper (1936) and Jürgens (1951) have shown that allyl formate and *N*-(diallylacetyl)urea, respectively give rise to hepatic cellular degeneration in experimental animals. *N*-(Allylisopropylacetyl)urea may cause thrombocytopenic purpura in humans (Ackroyd, 1949), may influence the course of human acute porphyria (Duesberg, 1932), and gives rise to the hepatic type of experimental porphyria in rabbits with the excretion of large amounts of uroporphyrin and porphobilinogen (Schmid & Schwartz, 1952). Diallylbarbituric acid has for some time been known to be one of the most toxic of the barbiturates and for this reason has generally been abandoned as an hypnotic in humans.

The finding in post-mortem tissue analysis that the liver and not the bone marrow contained excessive copro- and proto-porphyrin (and in one instance porphobilinogen) recalls the finding in the liver of porphobilinogen (Prunty, 1945) and uroporphyrin (Watson, Schwartz & Hawkinson, 1945)

in human acute porphyria and of these two substances in experimental porphyria in rabbits (Schmid & Schwartz, 1952). It also may be related to the known hepatotoxic effect of other allyl derivatives in experimental animals. It is of interest, too, that the coproporphyrin and uroporphyrin produced in excess were the series III isomers. Rimington (1952) has proposed a scheme of possible enzymic derangements, occurring in the porphyrias and the toxic porphyrinurias. It is not possible from the present results to interpret the precise mechanism occurring in the barbiturate porphyrinurias, but a reasonable inference from them might be that some of the barbiturates, especially those containing the allyl group, may interfere with the enzymes which decarboxylate uroporphyrin III to coproporphyrin III and protoporphyrin and, perhaps, with those responsible for the transformation of protoporphyrin to haem. The main site of this interference may possibly be the liver.

The present findings raise the questions of the bearing of these animal experiments upon barbiturate medication in human beings and of the relation, if any, which exists between human acute porphyria and the disturbances of pigment metabolism caused by barbiturates in rabbits. The doses of barbiturates used in the animal experiments were proportionately (weight for weight) greatly in excess of those normally taken by humans. The rabbits, however, generally tolerated these daily doses well and were mostly able to survive the period of drug administration. Humans if given a proportionate amount of barbiturate almost certainly could not survive. A weight-for-weight comparison of the effect of the barbiturates on human and rabbit porphyrin metabolism would therefore be misleading. From these experiments, we may possibly infer that barbiturates in proportionately smaller dosage may affect the abnormally sensitive porphyrin metabolism in human acute porphyrias; the clinical impression that this does in fact take place may receive some substantiation from these results. The differing degree of effectiveness of the barbiturates in the experimental animal may also explain the divergence of opinion on this clinical impression, which was based on the observation of different barbiturates. It is of great interest that diallylbarbituric acid administered to a normal animal causes the excretion of porphobilinogen and uroporphyrin.

A further point of inference may be important. Barbiturates have been shown to inhibit some of the oxidative processes of brain tissue (Quastel & Wheatley, 1933), the degree of inhibition being directly proportional to the hypnotic activity of the drugs. These authors also emphasized the importance of the allyl group in this connexion. Changes in

nerve and brain tissue occurring in acute porphyria (Mason, Courville & Ziskind, 1933; Denny-Brown & Sciarra, 1945) may be the most important lesions responsible for the clinical results of this disease. It is possible that barbiturates might aggravate this upset in brain and nerve metabolism, as distinct from their effect on porphyrin metabolism. This might explain why some barbiturates, producing no marked effect on the porphyrin metabolism of rabbits, have been among those suspected of adversely affecting the course of acute porphyria.

SUMMARY

1. Barbiturates have been suspected of adversely affecting the clinical course of acute porphyria. To assess this clinical impression objectively, the effect of nine barbiturates on the porphyrin metabolism of rabbits has been observed.

2. These barbiturates may be classified into four groups. (i) Diallylbarbituric acid, which caused a considerable rise of urinary coproporphyrin III in each of eight experimental rabbits and in three of these the excretion also of uroporphyrin III and porphobilinogen. (ii) Allylisopropylbarbituric acid and sodium allyl(1-methylbutyl)barbiturate, which caused a moderate rise of urinary coproporphyrin III. (iii) Sodium diethylbarbiturate, sodium ethyl(1-methylbutyl)barbiturate and sodium ethylphenylbarbiturate, which caused only a slight coproporphyrin increase in the urine. (iv) Sodium ethyl(1-methylbutyl)thiobarbiturate, sodium isomylethylbarbiturate and sodium butylethylbarbiturate, which did not alter significantly the urinary coproporphyrin excretion.

3. The effect of the presence of an allyl group in the barbiturate is discussed.

4. The significance of these results in animals is considered in relation to human acute porphyria.

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REFERENCES

- Ackroyd, J. F. (1949). *Clin. Sci.* **7**, 249.
 Chu, T. C., Green, A. A. & Chu, E. J. (1951). *J. biol. Chem.* **190**, 643.
 Datta, S. P., Dent, C. E. & Harris, H. (1950). *Science*, **112**, 621.
 Denny-Brown, D. & Sciarra, D. (1945). *Brain*, **68**, 1.
 Discombe, G. & D'Silva, J. L. (1945). *Brit. med. J.* **ii**, 491.
 Dobrschansky, M. (1906). *Wien med. Pr.* p. 2144.
 Duesberg, R. (1932). *Münch. med. Wschr.* **79**, 1821.
 Eliaser, M. & Kondo, B. O. (1942). *Amer. Heart J.* **24**, 696.
 Falk, J. E. & Benson, A. (1953). *Biochem. J.* **55**, 101.
 Günther, H. (1922). *Ergebn. allg. Path. path. Anat.* **20**, 608.
 Haxthausen, H. (1927). *Derm. Wschr.* **84**, 827.
 Jørgensen, J. & With, T. K. (1947). *Lancet*, **i**, 54.
 Jürgens, R. (1951). *Arch. exp. Path. Pharmacol.* **212**, 440.
 Laubender, W. & Monden, K. (1938). *Arch. exp. Path. Pharmacol.* **188**, 562.
 Lehmann, F. & Zinn, W. (1910). *Berl. klin. Wschr.* **47**, **ii**, 2244.
 MacLean, I. C. (1912). *Lancet*, **i**, 647.
 Mason, R., Courville, C. & Ziskind, E. (1933). *Medicine, Baltimore*, **12**, 355.
 Nicholas, R. E. H. (1951). *Biochem. J.* **48**, 309.
 Nicholas, R. E. H. & Rimington, C. (1951). *Biochem. J.* **48**, 306.
 Popper, H. (1936). *Virchows Arch.* **298**, 574.
 Prunty, F. T. G. (1945). *Biochem. J.* **39**, 446.
 Prunty, F. T. G. (1946). *Arch. intern. Med.* **77**, 623.
 Quastel, J. H. & Wheatley, A. H. M. (1933). *Proc. Roy. Soc. B*, **112**, 60.
 Rimington, C. (1942). *Brit. med. J.* **i**, 177.
 Rimington, C. (1952). *Acta med. scand.* **143**, **III**, 161.
 Rimington, C. & Sveinsson, S. L. (1950). *Scand. J. clin. Lab. Invest.* **2**, 209.
 Rommel (1912). *Charité-Ann.* **36**, 62.
 Rosendorff, W. (1910). *Berl. klin. Wschr.* **47**, **(i)**, 934.
 Schmid, R. & Schwartz, S. (1952). *Proc. Soc. exp. Biol., N.Y.*, **81**, 685.
 Sowden, G. S. (1910). *Brit. Med. J.* **ii**, 140.
 Turner, W. J. (1938). *Arch. intern. Med.* **61**, 762.
 Vahlquist, B. (1939). *Hoppe-Seyl. Z.* **260**, 189.
 Waldenström, J. (1939). *Acta psychiat., Kbh.*, **14**, 375.
 Waldenström, J. (1940). *Svenska Läkartidn.* **37**, 1537.
 Watson, C. J., Schwartz, S. & Hawkinson, V. (1945). *J. biol. Chem.* **157**, 345.