of view of breed of animal, time of year when it appeared, effect of diet, etc. When the urine was stored for some time after collection the 'cow-spot' again disappeared and the glycine spot only was present.

DISCUSSION

Although many individual tissues have been examined for their free amino-acids only two papers have come to the notice of the author where several different tissues of any one animal have been studied. The paper by Awapara *et al.* (1950) on the free amino-acids of rat tissues includes many results which are at variance with the work reported here. It is unnecessary to discuss all the differences, but it may perhaps be relevant to mention the most widely divergent results. Awapara *et al.* (1950) detected no serine, valine, lysine, histidine or carnosine in muscle. They did, however, find methionine and hydroxyproline. It is noticeable that they detected no leucines in any tissue, but found methionine in all of them. One may suppose that they have mistaken the leucine spot for methionine, for both amino-acids run to almost identical positions on a phenol-collidine chromatogram. The methionine can only be definitely identified by oxidizing it to its sulphone (Dent,

1948). Awapara *et al.* also state in their paper that the appearance of β -alanine and histidine upon acid hydrolysis of liver and skeletal-muscle extracts points to the existence of carnosine in these organs. It is possible that on the chromatograms of the unhydrolysed extract they have mistaken the carnosine for hydroxyproline since both give brownish yellow spots with ninhydrin, though the carnosine spot will go green in colour if heated to 105° (Dent, 1948).

The analyses of Gordon (1949) of ox muscle and ox serum agree quite satisfactorily with the results obtained in the present work. Arginine, tyrosine and phenylalanine were, however, not detected in the concentration of muscle extract applied to the chromatograms. Gordon also found tryptophan in the ox serum which he examined. This amino-acid was not recognized in the present work in any tissue. It is possible that loss of tryptophan occurred at some stage during the preparation of the extract.

SUMMARY

The free amino-acids occurring in various tissues and body fluids of the rat and cow have been detected using the technique of paper partition chromatography.

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The Fate of Intravenously Administered Coproporphyrin III in Normal and Lead-treated Rabbits

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Abnormally large amounts of coproporphyrin, mainly of type III, are usually found in the urine of mammals to which salts of lead have been administered. A three-carboxyl porphyrin has also been observed (Nicholas & Rimington, 1951; Weatherall & Comfort, 1952), and the latter authors have found evidence of a porphyrin containing five carboxyl groups. The quantity of coproporphyrin in the

faeces is little or not at all increased, at least in man (Kark & Meiklejohn, 1941) and rabbits (Fischer & Duesberg, 1932; Weatherall, 1948 and unpublished observations; Schwartz & Zagaria, 1951), and the coproporphyrin which is present is predominantly of type I (Dobriner, 1937*a*; Vigliani & Libowitzky, 1937; Watson, 1937), as in normal subjects. The abnormal excretion of coproporphyrin is therefore

more or less confined to the urine. This is surprising, because injected coproporphyrin is normally mainly excreted in the bile (Dobriner, 1937*b*; Vigliani, Angeleri & Sano, 1938). Even in lead-poisoned patients only a small fraction of injected coproporphyrin can be accounted for in the urine (Vigliani, 1938). Earlier studies (Fischer & Hilmer, 1926; Grotepass & Hulst, 1935) suggesting that administered coproporphyrin is excreted by the kidneys lack sufficient detail to provide reliable evidence on the problem.

A possible explanation of these observations is that the coproporphyrin recovered from the urine is excreted not as such, but as a precursor, which changes to coproporphyrin after it has passed the kidney. Various observations, summarized by Watson, de Mello, Schwartz, Hawkinson & Bossenmaier (1951), show that at least part of the urinary coproporphyrin is excreted in this way, and that the precursor changes to the free porphyrin very easily during extraction. Watson *et al.* (1951) extracted rapidly fresh samples of urine from various sources and found up to two-thirds of the total coproporphyrin present as a precursor. Eriksen (1951) reported similar findings. Neither Watson nor Eriksen excluded the possibilities that conversion of the precursor could occur while the urine was accumulating in the bladder, or after collection and during their extractions. If the one-third of the coproporphyrin recovered which is supposedly 'preformed' had in fact developed during this time, it would have been in keeping with their observations.

If all the urinary coproporphyrin is excreted as a precursor, the discrepancy between the normal biliary excretion of injected coproporphyrin and the appearance of coproporphyrin in the urine in lead poisoning would be explained. The abnormal metabolite in animals exposed to lead would then be the precursor and not coproporphyrin itself. To examine this hypothesis, some observations have been made on the precursor in the urine of rabbits treated with lead. Also, coproporphyrin III has been administered to normal and to lead-treated rabbits and the amounts found in the urine and bile have been estimated.

METHODS

Administration of lead salts to rabbits. Male chinchilla rabbits weighing 1.7–2.5 kg. were used in all the experiments. They received an initial dose of PbCl_2 intravenously (0.05 m-mole/kg. given in 0.02 M solution at a rate of 2.0 ml./min.) and after a fortnight they were given a diet to which 250 parts of Pb, as PbCl_2 , was added per million parts by weight of diet. This is roughly equivalent to a daily intake of 0.2 mg. Pb/kg. body weight. The duration of poisoning has been measured from the time of the priming dose. After some initial depression, the rabbits remained apparently well on this treatment, but excreted abnormally large amounts of coproporphyrin.

Cannulation of the bile duct. Rabbits were anaesthetized with pentobarbitone (Nembutal, Abbott) (25–40 mg./kg. intravenously initially, supplemented with smaller amounts or with ether by inhalation as required). The cystic duct was tied and the common bile duct was cannulated with a small glass cannula and the bile was collected in graduated tubes. Artificial respiration was given if necessary, though this was unusual. Blood pressure was recorded with a mercury manometer from the carotid artery. Bile flowed freely only when the blood pressure was above 60–70 mm. Hg. and nearly all the rabbits were given ephedrine hydrochloride (5 mg./kg. intravenously, repeated if necessary) and 0.9% (w/v) NaCl or 6.0% dextran in 0.9% NaCl (Dextran-Benger) to maintain their blood pressure above this level.

Extraction of coproporphyrin and its precursors. Coproporphyrin was extracted from urine and bile by the usual acetic acid-ether procedure, with one of two variants, called A and B below. In both procedures, except when otherwise stated, samples of 5–15 ml. of urine or bile were extracted twice with 15 ml. of ether. Glacial acetic acid (2–5 ml.) was added to the first portion of ether before extracting. The two ethereal extracts were combined and washed with 5 ml. 0.015 M-sodium acetate. In procedure A the ether was then extracted immediately with N-HCl until no more material fluorescing red or pink in ultraviolet light was obtained. In procedure B, the ether was washed once with 0.005% (w/v) aqueous I_2 before extraction with N-HCl (cf. Schwartz, Zieve & Watson, 1951). In each procedure the pH of the solution in HCl was raised to about 4, as judged by bromophenol blue as external indicator, with solid or a saturated solution of sodium acetate. The porphyrins were extracted with two or more 10 ml. portions of ether and from the ether with 0.07 N-HCl. Extracts were taken until they did not fluoresce red in ultraviolet light. In procedure A, all the portions of ether used in the extractions were kept on the bench for 18–24 hr. and again extracted with N-HCl. In this time red-fluorescent material commonly appeared, and occasionally still more was found after standing for another day. These extracts were neutralized and taken via ether to 0.07 N-HCl as before, and were estimated separately. The first portion included any coproporphyrin which had been extracted as such and an uncertain amount formed from precursor. The later portions contained only porphyrin formed from precursor after excretion. In some experiments, performed before the importance of the precursor was recognized, the ether was not kept and re-extracted and only the equivalent of the first portion was taken. This procedure is referred to as A-. When procedure B was used and the various portions of ether were kept as in procedure A, no additional red-fluorescent material developed on standing which could be extracted with HCl; it was therefore assumed that all the precursor in the sample was converted to free porphyrin by the wash with I_2 . Extracts in 0.07 N-HCl prepared from bile were washed with 0.5 vol. of CHCl_3 twice, or more often if any trace of blue or green pigments was observed in the first wash before estimation. The porphyrins in 0.07 N-HCl were estimated in a Unicam spectrophotometer (λ , 401 $\text{m}\mu$.) calibrated with a standard solution of coproporphyrin in the same solvent.

The extracts of 0.07 N-HCl were liable to contain porphyrins believed to have three and five carboxyl groups as well as coproporphyrin (Weatherall & Comfort, 1952). Their contribution to the total absorption at 401 $\text{m}\mu$. has not yet been studied, and has been ignored as they are only a small fraction of the total porphyrin present. In order to detect

them, samples of the porphyrin after estimation were adsorbed on to acid-washed tale (Nicholas & Comfort, 1949), eluted with 0.5N-NH₄OH in acetone and chromatographed on paper at 7° with 40% (v/v) water in lutidine (Nicholas & Rimington, 1949, 1951) in parallel with standard coproporphyrin or deuteroporphyrin.

Preparation of coproporphyrin. Urine from rabbits to which PbCl₂ had been administered was collected from galvanized-metal metabolism cages and was extracted as indicated above, except that larger volumes were used. The ether-soluble porphyrins so obtained were taken into ether and then to dryness. They were esterified with methanol saturated at 0° with dry HCl gas; dissolved in CHCl₃; washed repeatedly with 0.1M-Na₂CO₃ and then with water; and chromatographed from benzene on Al₂O₃ (Nicholas, 1951). On developing the chromatograms with increasing concentrations of CHCl₃ in benzene, several fractions appeared. Details will be reported separately. The main porphyrin present was eluted with 30% (v/v) CHCl₃ in benzene, and was crystallized from CHCl₃ and light petroleum. The needle-shaped crystals appeared typical of coproporphyrin III and melted at 146°, remelting at 164° (uncorr.). A standard solution of coproporphyrin was prepared by leaving a weighed quantity (2 mg.) of the methyl ester in 7.5N-HCl for 36 hr. at room temperature and then diluting with water to 0.6N and storing in the refrigerator. The identity of the porphyrin was checked by its absorption spectra in ether and in HCl and by paper chromatography, when it ran as a single spot (*R_F*, about 0.65) in parallel with a sample of coproporphyrin kindly presented by Prof. C. Rimington and Miss R. E. H. Nicholas. For injection into rabbits the solution was nearly neutralized with 2.5N-NaOH and made faintly alkaline with 0.5M-NaHCO₃ immediately before use; it was diluted with water until it was approximately isotonic with 0.15M-NaCl.

RESULTS

The presence of a precursor of coproporphyrin in urine

Experiments similar to those described by Watson *et al.* (1951) were performed on urine obtained immediately before extraction from lead-treated rabbits by suprapubic pressure. As exposure to ultraviolet light accelerates conversion of the precursor (Saillet, 1896; Watson *et al.* 1951), the experiments were done in a dark room lit by one 25 W. electric lamp about 1.7 m. from the work bench. Urine (5 or 10 ml.) was extracted once with 5–10 vol. of ether or ethyl acetate, so that foams did not form and separation occurred rapidly. The organic solvent was washed once with 0.015M-sodium acetate and the free porphyrin was extracted with 4 × 2 ml. N-hydrochloric acid. Other experiments on comparable samples of urine showed that this removed all the initially detectable red-fluorescent material. The organic solvent was washed with 0.005% (w/v) iodine to convert any precursor remaining in it and was again extracted with N-hydrochloric acid. When the first set of extractions with N-hydrochloric acid was completed within

10 min. of the urine being obtained from the bladder, and the additional material obtained after washing the organic solvent with iodine was estimated separately, the mean amount of apparently preformed porphyrin was 51% (s.d. of nine observations on different samples of urine ± 19%) and the minimum 17%. When the extraction was performed more slowly, the amount of apparently preformed porphyrin was greater. The mean increment in samples extracted in 30 instead of 10 min. was 9% and in samples extracted in 90 min. was 21%. As expected, therefore, conversion of precursor was occurring in the conditions of these experiments and the estimates of preformed porphyrin are likely to be too large. Simple attempts to prevent the conversion were unsuccessful. It was impracticable to perform the extractions much more rapidly. The addition of reducing agents, such as ferrous sulphate, ascorbic acid or thioglycolic acid (all 0.01M) to the urine had no substantial effect, nor did the use of ice-cold reagents. As the identity and exact behaviour of the precursor of coproporphyrin are not known, it is possible that the procedure here described did not extract all that was present. Also, some is likely to have been converted to coproporphyrin before the extraction of the latter was completed. Both sources of error tend to overestimate the proportion of preformed porphyrin. The present findings therefore confirm previous observations that over half the urinary coproporphyrin is liable to be excreted as a precursor; they are compatible with the thesis that all the urinary porphyrin is excreted in this way and part of it is converted to free porphyrin too rapidly to be recognized as precursor.

Biliary excretion in anaesthetized rabbits

The results of experiments in which the biliary and, in most cases, the urinary excretions of coproporphyrin were measured are shown in Table 1. Most of the experiments were performed before the importance of the precursor was recognized. The extractions were therefore performed by method A–, but they were carried out particularly slowly, in the intervals of attending to the experimental animal, so that most of any precursor present probably changed to the free porphyrin and was recovered as such. In later experiments, including those on rabbits 103 and 116 shown in the table and some on bile obtained from other anaesthetized rabbits, the full extraction procedure A was followed. When bile was extracted, a little additional porphyrin was always found in the ether allowed to stand until the following day, but in no case did the amount recovered represent more than an additional 0.5 µg./hr. It does not therefore appear that any serious error arose from the neglect of the unconverted precursor in the earlier experiments. In

Table 1. *Biliary and urinary excretion of coproporphyrin III after intravenous injection into anaesthetized rabbits*

(In these experiments coproporphyrin was extracted by procedure A -.) (N.D. Not done. N.U. No urine found in bladder.)

Treatment	...	...	...	Normal						Lead-treated							
Duration of lead treatment (days)	...	...	...	—	—	—	189	94	89	323							
Rabbit no.	...	...	...	111	113	116	78	98	99	103							
Weight (kg.)	...	...	...	2.0	1.9	1.8	2.1	1.9	1.7	2.3							
Coproporphyrin III (100 μg.) injected at beginning of 3rd hr.	...	...	...	No	Yes	Yes	No	Yes	Yes	Yes							
				(ml.) (μg.)		(ml.) (μg.)		(ml.) (μg.)		(ml.) (μg.)		(ml.) (μg.)		(ml.) (μg.)		(ml.) (μg.)	
Bile vol. and coproporphyrin content:																	
1st hr. of collection				10.5	N.D.	10.3	1.3	10.4	1.1	7.3	1.1	7.2	1.0	9.3	4.8	6.1	1.1
2nd hr. of collection				7.2	2.6	7.9	1.3	4.8	0.2	6.5	1.3	6.0	0.4	4.2	5.1	6.5	1.0
3rd hr. of collection				7.4	N.D.	5.9	25.5	5.6	20.8	6.0	1.4	4.9	17.8	3.8	20.0	9.2	36.0
4th hr. of collection				7.9	1.4	6.4	19.6	3.8	20.3	6.9	2.1	3.8	9.0	3.1	15.9	9.4	27.6
5th hr. of collection				7.9	N.D.	6.1	21.0	0.5	1.2	8.2	1.6	3.1	6.8	Died		8.4	10.3
6th hr. of collection				3.4	1.0	5.9	14.0	0.6	1.1	11.7	1.7	2.2	4.3			8.2	6.5
Total in 6 hr.				44.3	10.0*	42.5	82.7	25.7	44.7	46.6	9.2	27.2	39.3	(20.4) (45.8)		47.8	82.5
Urine vol. and coproporphyrin content:																	
Total in 6 hr.				N.D.	37	7.5	39	1.7	20	27.0	N.D.			N.U.		9	25.0

* Estimated from average of available results.

order that the results may be comparable, even when additional precursor was looked for, only the quantities obtained in the first portion are given in Table 1.

Coproporphyrin was administered intravenously 2 hr. after beginning the collection of bile. An estimate of the normal biliary excretion of coproporphyrin under the conditions of these experiments can be based on all the results obtained in the 2 hr. before coproporphyrin was given. For five normal rabbits the mean excretion was 1.3 ± 0.6 (s.e.) µg./hr. and, for six lead-treated rabbits, 1.7 ± 1.1 (s.e.) µg./hr. (These figures include values for some rabbits not shown in Table 1.) This difference could easily occur by chance ($t = 0.42$; $0.7 > P > 0.6$). The samples are small, but show that there is not a consistently increased amount of coproporphyrin in the bile of lead-poisoned rabbits comparable to the greatly increased urinary coproporphyrin.

When no coproporphyrin was administered, the rate of coproporphyrin excretion in the bile remained fairly steady. When coproporphyrin (100 µg.) was injected intravenously at the beginning of the third hour, the rate of excretion in the bile rose to 18–36 µg./hr. and then declined gradually, so that 35–75 % of the administered material was accounted for in the 4 hr. after injection. This

was equally true of the normal and the lead-treated rabbits. In the two normal rabbits which survived for the whole period, 83 and 45 µg. respectively of coproporphyrin were recovered from the bile and in the two which had received lead, 83 and 39 µg. No excess of coproporphyrin, other than that expected in lead-treated animals, was found in the urine of any of the rabbits. While the close similarity of the biliary figures is presumably fortuitous, there is no evidence of an inability of the liver to excrete the administered material, or of a difference in the fate of coproporphyrin in the normal and the lead-treated rabbit.

All the porphyrins recovered from bile (from normal and lead-treated rabbits before and after coproporphyrin was injected) ran on paper with the same R_f as standard coproporphyrin III. The amounts present before coproporphyrin was injected were so small that if three- and five-carboxyl porphyrins had been present in the same proportion to coproporphyrin as they have been found in the urine of lead-treated rabbits, they would almost certainly not have been detected. The porphyrins recovered from the urine of these rabbits also ran on paper as coproporphyrin, and a trace of a three-carboxyl porphyrin was found in the urine of lead-treated rabbit 103.

Recovery of coproporphyrin from the urine after intravenous injection into unanaesthetized rabbits

In order to exclude abnormalities due to the anaesthesia and the operative procedure of the experiments on biliary excretion, coproporphyrin (100 μ g.) was administered also to two conscious normal rabbits and to two conscious lead-treated rabbits. In order to obtain a free flow of urine, water was given by stomach tube in two doses of 50 ml./kg. 1 hr. apart and 1–2 hr. before injecting the coproporphyrin. The experiment was performed on a cross-over design, one rabbit of each pair receiving coproporphyrin and the other 0.15M-sodium chloride on one day and vice versa on another day 3 or more days later. Urine was collected by suprapubic pressure at 30 min. intervals during the diuresis, but no appreciable difference was observed in the rate of flow of urine with or without administered coproporphyrin. In the normal rabbits there was no increase in the amount of coproporphyrin in the urine after its administration (Table 2). In the lead-treated rabbits the irregular but high rate of excretion made large differences in the total amount recovered insignificant. One rabbit (126) excreted less coproporphyrin in the 24 hr. after its administration than after the administration of saline, and the other rabbit (140)

excreted more. However, the latter rabbit had been excreting still higher amounts of coproporphyrin in the 2 days immediately before the coproporphyrin was injected (996 μ g. in 48 hr.), so the results do not suggest that there was any substantial urinary excretion of the administered porphyrin. Faeces were collected from these rabbits for 3 days after the injections. In each case more coproporphyrin was found in the faeces of the rabbit which had received coproporphyrin, but the quantities of faeces varied so greatly that the significance of this finding is doubtful.

In addition, coproporphyrin III was administered to some normal and some lead-treated rabbits without additional water, and the urine was collected over periods of 3 days. The amounts of porphyrin recovered did not show any increase after coproporphyrin was injected. The mean output of the normal rabbits in these experiments was unusually low (less than 1–2 μ g./day) and it is possible that the porphyrin was decomposing during the period of collections: Schwartz *et al.* (1951) have shown that this occurs in acid urines, and the pH of these samples was not recorded. It is unlikely that large differences in urinary coproporphyrin concentration were obliterated by such deterioration, so the experiments provided some confirmation of the results obtained during diuresis on fresh samples.

Table 2. *Urinary volume and coproporphyrin content after intravenous injection of coproporphyrin III into unanaesthetized rabbits*

(Coproporphyrin was extracted by procedure B.)

Treatment						Normal rabbits							
Material injected						Saline				100 μ g. coproporphyrin			
Rabbit no.						113	114	113	114	113	114	113	114
Weight (kg.)						2.0	1.8	2.0	1.8	2.0	1.8	2.0	1.8
Experimental day						1	2	2	1	2	1	2	1
Urinary volume and coproporphyrin content:						(ml.)	(μ g.)	(ml.)	(μ g.)	(ml.)	(μ g.)	(ml.)	(μ g.)
0–4 hr. after injection						115	3.4	102	5.9	105	15.0	144	2.6
4–22 hr. after injection						151	8.8	115	18.0	97	8.3	115	8.5
Total						266	12.2	217	23.9	202	23.3	259	11.1
Treatment						Lead-treated rabbits							
Duration of lead treatment (days)						66–73							
Material injected						Saline				100 μ g. coproporphyrin			
Rabbit no.						136	140	136	140	136	140	136	140
Weight (kg.)						1.9	2.2	1.9	2.0	1.9	2.0	1.9	2.0
Experimental day						1	2	2	1	2	1	2	1
Urinary volume and coproporphyrin content:						(ml.)	(μ g.)	(ml.)	(μ g.)	(ml.)	(μ g.)	(ml.)	(μ g.)
0–4 hr. after injection						94	28	109	69	166	50	36	128
4–22 hr. after injection						78	131	25	112	4	18	69	216
Total						172	159	134	181	170	68	105	344

*Incidental observations on the effects of
administering coproporphyrin*

No abnormal behaviour was observed in the conscious rabbits after intravenous injection of 100 μg . of coproporphyrin. In the anaesthetized rabbits, there was usually a transient rise of blood pressure of 10–20 mm. Hg, which could be closely imitated by the injection of the same volume of 0.15M-sodium chloride solution at the same rate. In one rabbit (113) the responses of the blood pressure to adrenaline, acetylcholine and nicotine were examined before and twice after giving coproporphyrin, and showed no appreciable change. Intestinal motility was not visibly altered in one normal rabbit (not among those included in Table 1), in which the intestines were exposed during and after the porphyrin injection. No other effects were observed. These observations do not support the suggestion that coproporphyrin is responsible for some of the symptoms of lead poisoning (Schreus & Carrié, 1933), particularly as the doses used were a large fraction of the usual total daily excretion of lead-poisoned rabbits.

DISCUSSION

These observations on the precursor of coproporphyrin in the urine of rabbits treated with lead show that it behaves similarly to the precursor described by Sallet (1896) and others in other circumstances. Attempts to prevent the precursor from changing to the free porphyrin have not been effective and there is therefore no direct evidence about the proportion of the urinary coproporphyrin excreted as precursor. Reasons are given below for believing the proportion to be very high.

The value of the experiments in which coproporphyrin was administered to rabbits depends on the identity of the administered material and of the material subsequently recovered and estimated. The identification of the material which was injected depends on its source and method of isolation, on the appearance of crystals of its methyl ester and their melting point, on the chromatographic homogeneity of the ester and of the free porphyrin obtained by hydrolysis, and on the absorption spectrum of the porphyrin so obtained. All these indicate that it was predominantly coproporphyrin III, but do not completely exclude the presence of coproporphyrin I and conceivably of other as yet unrecognized porphyrins. A melting point of 143°, for instance, is consistent with the presence of up to about 10% of coproporphyrin I in the material (Jope & O'Brien, 1945). In view of the origin of the material there was unlikely to be more than this amount of the type I isomer present even before crystallizing the methyl ester (Schwartz & Zagaria,

1951), although some recent observations suggest that urine from humans exposed to lead may contain a higher proportion of coproporphyrin I (Kench, Lane & Varley, 1952). Porphyrins which were presumed to have three or five carboxyl groups were separated chromatographically, and no other ether-soluble porphyrins are known to occur in the urine in lead poisoning.

The identification of the material extracted from urine and bile and subsequently estimated, depends on the method of isolation, fluorescence, absorption spectrum and behaviour as a four-carboxyl porphyrin on paper chromatography. In all these respects it was identical with the material administered, so it seems probable that it was in fact the same substance.

In the experiments on biliary excretion the operative procedures and anaesthesia may have affected the metabolism of coproporphyrin. Under these conditions urine was being secreted and coproporphyrin was found in it in concentrations comparable to those observed without anaesthesia. There is therefore no reason to believe that the kidney was functioning abnormally in this respect. Bile was being secreted, and administered coproporphyrin appeared in it promptly and in high concentrations. Even if the administered coproporphyrin was distributed no more widely than in the plasma, its concentration would not have exceeded about 1 $\mu\text{g}/\text{ml}$., since the plasma volume in rabbits of the size used in these experiments is about 100–120 ml. (Courtice & Gunton, 1949; Armin, Grant, Pels & Reeve, 1952) and the dose given was 100 μg . Concentrations up to 5 times greater were found in the bile, so the liver was capable of concentrating coproporphyrin and it is unlikely that the operative procedures increased its ability to do so. Also, administered coproporphyrin did not appear in the urine whether the animals were anaesthetized or not, so it seems probable that in the conscious animal, also, the coproporphyrin was being excreted in the bile.

With allowance for the rate of secretion in animals to which no porphyrin was given, the quantity of coproporphyrin obtained from the bile varied from 35 to 75% of the quantity administered. At the end of the experiments the biliary excretion was still above its original level, so the whole dose might have been excreted in this way. It is also possible that a fraction of the dose was fixed or metabolized in the body, but there is no evidence that less was metabolized by the lead-treated rabbits.

Before any porphyrin was administered, the concentration of coproporphyrin in the bile was similar in normal and lead-treated rabbits. In each case the liver was able to remove coproporphyrin from the plasma, so it is probable that there was no excess

of coproporphyrin circulating as such in the plasma of the lead-treated rabbits. The excess of coproporphyrin found in the urine must therefore have been carried in the plasma in some other form which was secreted by the kidney and then changed more or less rapidly into coproporphyrin. The observations on the occurrence of a precursor are consistent with this hypothesis.

It follows that the precursor is not excreted to any great extent by the liver. If it was it would have been demonstrable as such or as an excess of coproporphyrin in the bile of lead-treated rabbits, and probably coproporphyrin III would be found in the faeces in lead poisoning. A little precursor was found in the bile in these experiments, and Watson *et al.* (1951) have reported its presence in human fistula bile; so some hepatic excretion is possible. Indeed, it is possible that all the coproporphyrin found in bile, as in urine, has been excreted as a precursor. This possibility does not affect the present argument, provided that the capacity of the liver to excrete precursor is limited.

If the present findings in rabbits and the interpretation placed on them may be extended to other species and other causes of increased coproporphyrin excretion, the amount of coproporphyrin in the urine reflects the concentration of circulating precursor, whereas surplus coproporphyrin as such in the circulation is removed by the liver and appears in the faeces, or at least in the bile. If this were so, shifts from urinary to faecal excretion would correspond to an increased formation of free coproporphyrin from precursor in the body. Obviously, considerably more evidence is desirable to justify such a generalization.

As far as lead poisoning is concerned, it appears that the metabolic abnormality is an over-production or under-utilization of the precursor or precursors of coproporphyrin III and not of coproporphyrin III itself. Progress on these lines depends on identifying the precursor, which has been suggested to be a metalloporphyrin (Gray, 1951; Eriksen, 1951) or reduced coproporphyrin or copro-

porphyrinogen (Watson *et al.* 1951). The experiments reported here do not throw fresh light on this question.

SUMMARY

1. Experiments have been performed to discover why coproporphyrin III appears in the urine, but not in the faeces, in lead poisoning.

2. It has been found that at least half the urinary coproporphyrin in lead-treated rabbits is formed from a precursor after the urine is passed.

3. Coproporphyrin III has been obtained from the urine of lead-treated rabbits, crystallized as its methyl ester, hydrolysed and injected in 100 μ g. doses intravenously into conscious and anaesthetized normal and lead-treated rabbits.

4. No additional urinary excretion of coproporphyrin III has been found.

5. In anaesthetized rabbits, the mean rate of excretion of coproporphyrin in the bile was 1.3 ± 0.6 (s.e.) μ g./hr. for normal rabbits and 1.7 ± 1.1 (s.e.) μ g./hr. for lead-treated rabbits. When 100 μ g. coproporphyrin were injected intravenously, this rose to 18–36 μ g./hr., and 35–75% of the dose was accounted for in the 4 hr. after injection. No difference was apparent between the biliary excretion of the normal and that of lead-treated rabbits.

6. It is suggested that the coproporphyrin found in the urine in lead-treated rabbits is excreted entirely as a precursor, and that the metabolic disorder produced by lead is an over-production or failure of utilization of a precursor of coproporphyrin and not of coproporphyrin itself.

7. The administered coproporphyrin had no effect on the behaviour, blood pressure or water diuresis of either normal or lead-treated rabbits.

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The Preparation and Properties of β -Glucuronidase

5. SPECIFICITY

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Knowledge of the specificity of the enzyme β -glucuronidase present in animal tissues is far from complete. Masamune (1934) found that the enzyme would hydrolyse neither *laevomenthyl* α -D-glucuronide nor a variety of α - and β -D-glucosides. Phenyl β -D-glucoside was slowly hydrolysed by Masamune's enzyme preparation, but Graham (1946) obtained no hydrolysis of this compound with highly purified ox-spleen β -glucuronidase. Karunairatnam (1950) found that phenyl β -D-glucoside was hydrolysed by mouse-liver homogenates, but that this property was lost in the early stages of purification of β -glucuronidase. Further information on the specificity of this enzyme, not recognized as such by the original authors, is provided by the work of Karunairatnam & Levy (1949) who, in their search for an efficient inhibitor, found that the rate of hydrolysis of phenyl β -D-glucuronide was unchanged in the presence of methyl α -D-glucoside, methyl α -D-mannoside, methyl β -D-glucoside, methyl β -D-xyloside and methyl α -D-galactoside. Had any of these compounds been substrates for the enzyme, a fall in the rate of liberation of phenol might have been expected.

Meyer, Linker & Rapport (1951) found that β -glucuronidase hydrolysed the oligosaccharides which are the end products of the action of purified cesticular hyaluronidase on hyaluronic acid. In the absence of adequate knowledge of the specificity of β -glucuronidase, this finding could not be put forward as additional proof of the structure of hyaluronic acid. Implicit in the use of β -glucuronidase as a hydrolytic agent in the study of urinary steroids is the assumption that all the steroid set free in this way is initially conjugated with glucuronic acid. Further information on its specificity would increase the value of β -glucuronidase as a biochemical reagent. The difficulty of preparing suitable test compounds has been largely overcome

by the development of a method for the oxidation of hexosides to hexuronides with gaseous oxygen in the presence of platinum catalyst (Marsh, 1952: cf. Fernandez-Garcia *et al.* 1950; Mehlretter, Alexander, Mellies & Rist, 1951; Barker, Bourne & Stacey, 1951). The opportunity has been taken to study the action of mouse-liver β -glucuronidase on α - and β -glucuronides, galacturonides and mannuronides, and on a variety of other compounds.

EXPERIMENTAL

Materials. The following compounds were synthesized by methods given in the literature and were converted into the corresponding conjugated hexuronic acids (Marsh, 1952): (-)-menthyl α -D-glucopyranoside, methyl α - and β -D-galactopyranosides, methyl α - and β -D-mannopyranosides, and β -D-glucopyranose-1-phosphate. Biosynthetic α -D-glucopyranose-1-phosphate, presented by Dr R. J. Whelan, was likewise put through the oxidation procedure. It should be noted that β -D-glucuronic acid-1-phosphate has not been obtained pure. The glucuronic acid content of the specimen used here was 60% of theory for the tripotassium salt.

Methyl α -D-glucopyranoside, phenyl β -D-glucopyranoside and (-)-menthyl β -D-glucopyranoside were also prepared by standard methods. Methyl α - and β -D-glucopyranuronides were obtained in solution by treating the corresponding 3:6-lactones with dilute NaOH. Methyl 4-methyl-D-glucopyranuronide (mixture of α and β forms) was obtained in solution from the amide by heating the latter with NaOH (Smith, 1951). This amide and the two glucopyranuronolactones were presented by Prof. F. Smith. Oxycellulose (polyanhydroglucuronic acid, Yaackel & Kenyon 1942) was presented by Imperial Chemical Industries, Ltd. (Explosives Group). It had a COOH content of 22% (theoretical value for polyanhydroglucuronic acid, 26%), and was brought into solution by careful addition of dilute NaOH, so that the pH never exceeded 7.

The standard substrates, phenolphthalein, phenyl and (-)-menthyl β -D-glucuronides, were biosynthetic.

Enzyme preparation. Unless stated to the contrary, the enzyme was prepared as follows. Mouse liver (1-2 g.) was homogenized in water and made up to a final volume of 9 ml.