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EXPERIMENTAL PORPHYRIA IN RATS INDUCED BY CHLORINATED BENZENES

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Abstract—Like hexachlorobenzene, the lower chlorinated benzenes are able to induce experimental hepatic porphyria in rats. Feeding of mono-, di-, tri and tetrachlorobenzenes to male albino rats was followed first by increased urinary coproporphyrin excretion, later by rise in porphobilinogen and δ-aminolaevulic acid. There are differences in the excretory pattern between animals treated with lower chlorinated benzenes and Sedormid or allylisopropylacetamide (AIA). Liver GSH content was found to be reduced in starved rats after administration of 1, 2, 4-trichlorobenzene. GSH given to rats, highly porphyric due to 1,2,4-trichlorobenzene-intoxication, had a protective effect. Preliminary investigations were made of hepatic pyridine nucleotide levels in rats made porphyric by intoxication with chlorinated benzenes, AIA or Sedormid. Some deviations from normality were observed. Hair loss was noted due to follicular hyperkeratosis similar to the acneiform eruptions seen in man due to chlorinated compounds. The experimental animals showed no signs of photosensitivity.

A WIDESPREAD outbreak of human cutaneous porphyria in Turkey was in 1959 traced to the ingestion of wheat treated with a fungicide containing hexachlorobenzene.[1–3] Hepatic porphyria was later shown to be produced in rats and rabbits by feeding hexachlorobenzene.[4, 5] Since the other chlorinated benzenes are also used industrially for a wide variety of purposes it was thought of importance to investigate the effects on porphyrin metabolism in rats produced by feeding these substances in the diet. As will be shown, all of the chlorinated benzenes produce disturbances in porphyrin metabolism similar to that produced by hexachlorobenzene. The dose required and the severity of the ensuing disturbance vary with the compound being studied.

METHODS

Male albino rats (body wt. 150–250 g) were fed standard Medical Research Council diet 41B and placed in metabolic cages allowing separated collection of urine and faeces. After a preliminary period of 3 days for base-line determinations, they were fed the drug under investigation daily by gastric intubation. The drug was supended either in 1% cellofas (Imperial Chemical Industries, Ltd.), liquid paraffin, or in propylene glycol (Drug conc. 100–200 mg/ml). The chlorinated benzenes were obtained from the following sources: monochlorobenzene and *p*-dichlorobenzene from Griffin and George, Ltd.; *o*-dichlorobenzene from Fluka, A.G., Switzerland; 1,2,3-trichlorobenzene, 1,2,4-trichlorobenzene and 1,2,4,5-tetrachlorobenzene from British Drug Houses, Ltd.; 1,2,3,4-tetrachlorobenzene was kindly provided by Dr. Gage of Imperial Chemical Industries. 2-*Allyl*-2-*iso*propylacetamide (AIA) and 2-*iso*propylpent-4-enoylurea

(Sedormid) were obtained from Hoffmann-La Roche, Ltd. All reagents used in the estimations were of AnalaR quality.

Determination of porphyrins

Urine. Urinary porphyrins were determined on 24-hr samples. Coproporphyrin was extracted from the urine by the method of Rimington;[6] care was taken to shake back any coproporphyrin present in the iodine and water washings. The method chosen for the determination of uroporphyrin extraction depended on the amount of pigment present in the urines. For urines with low uroporphyrin content, extractions were carried out with ethyl acetate at pH 3–3·2;[7] for higher concentrations of uroporphyrin, the *cyclo*hexanone extraction of Dresel, Rimington and Tooth[8] at pH 1·5 was employed. The extinction of each acid extract in 5% HCl (w/v) was determined at 380, 340 mμ, and at the Soret maximum using 1-cm path-length cells and a Unicam Spectrophotometer SP 500. Porphyrin contents were calculated from the extinction readings using the correction formulae of Rimington and Sveinsson[9] and the extinction coefficients given by Rimington.[6]

Liver. Liver (4–5 g) was homogenized with cold water to give a 1:5 tissue suspension; this suspension was shaken vigorously with 10 volumes of a mixture of ethyl acetate and glacial acetic acid (4:1) and the resultant mixture was left over-night at 4°; the subsequent extraction of porphyrin was as described by Dresel and Falk.[10] In the estimation of porphyrin, the extinction of each acid extract was measured at 380 mμ and 430 mμ, and at the Soret band maximum. Porphyrin contents were calculated per 100 g wet wt. using the correction formulae of Rimington and Sveinsson.[9] Since uroporphyrin is adsorbed to a certain extent on the precipitated protein, values are low and Dresel and Falk[10] found a recovery of approximately 50 per cent for added uroporphyrin with the above procedure. We have, therefore, multiplied the uroporphyrin values found by a factor 2 to correct for this adsorption, so obtaining a rough estimate of the uroporphyrin content of the liver suspensions.

Paper Chromatography. The chromatographic separation and identification of coproporphyrins I and III was done using the ascending 2,6-lutidine–water method.[11]

Determination of porphyrin precursors

Urinary porphobilinogen (PBG). Porphobilinogen was estimated on fresh 24-hr urine samples by the method of Mauzerall and Granick[12] using Dowex 2-X8 resin, with the slight modification that in the case of urines with small PBG contents the acetic acid eluates were diluted to a final volume of 5 ml with water.

Urinary δ-aminolaevulic acid (ALA). The method for estimating ALA of Mauzerall and Granick[12] was modified for use with Dowex 50 W-X8 resin. The combined aqueous and urinary eluates from the Dowex 2-X8 used in the method for PBG mentioned previously were transferred to a Dowex 50 W-X8 resin; urea was removed by washing twice with 10 ml water. ALA was then eluted with 9·5 ml 0·5 M sodium acetate and the eluate was made up to 10 ml by acetate buffer pH 4·6. In a separate tube 0·3 ml acetylacetone was mixed with 3 ml of the same buffer and this mixture added to the ALA-containing solution. After mixing well, the solution was heated at 100° for 10 min, cooled and to 2 ml was added 2 ml of the Ehrlich reagent described by Mauzerall and Granick,[12] and the optical density read at 553 mμ after 15 min. The blank was

prepared by mixing 10 ml 0·5 M sodium acetate with 0·3 ml acetylacetone and 3 ml of the acetate buffer and mixing 2 ml of this with 2 ml Ehrlich reagent. The optical density observed multiplied by 62 = μg ALA per ml of original urine. Recoveries of ALA by this method were 98–100 per cent within the range 10–1000 μg/ml.

Porphobilinogen in liver. Six millilitres of a 1:5 liver suspension in water were mixed with 2 ml trichloroacetic acid–mercuric chloride solution (4 parts of 20% (w/v) trichloroacetic acid plus one part 0·25 M mercuric chloride). After vigorous shaking and centrifuging, the supernatant was treated with an equal volume of modified Ehrlich reagent for the estimation of PBG as described by Mauzerall and Granick.[12]

Liver catalase determination. Catalase activity in liver tissue was determined by the method of Feinstein;[13] the results were calculated as milli-equivalents of sodium perborate destroyed per mg of liver wet wt. Normal untreated male rats gave a mean value of 0·85 m-equiv./mg wet wt.

Liver ALA-dehydrase determination. Estimations of ALA-dehydrase in liver tissue were carried out by the anaerobic method of Gibson, Neuberger and Scott[14] with an incubation time of 1 hr at 38° after tipping the ALA into the reaction mixture. Porphobilinogen formed as a result of the reaction was estimated by the method of Mauzerall and Granick[12] modified as described above and the activity of the enzyme expressed as mols of PBG produced per g liver wet wt. under the conditions described. Normal untreated male rats gave a value of 1·1 mols PBG/g liver wet wt.

Liver glutathione determinations. Glutathione was determined both spectrophotometrically and iodometrically by the methods described by Grunert and Phillips[15] and Woodward and Fry[16] respectively. For these estimations, animals were starved for 24 hr prior to dosing with 500 mg/kg of the chlorobenzene under investigation and were denied water for the last hour before sacrifice. Animals were killed 2 hr after dosing with the drug; these estimations were kindly carried out by Mr. C. W. I. Owens.

Liver nucleotide determinations. Determinations of NAD, $NADH_2$, NADP and $NADPH_2$ in animals treated with chlorinated benzenes were kindly performed by Dr. T. F. Slater. The method of estimation was as described by Slater and Sawyer.[17]

Light sensitivity test. Skin tests were kindly carried out by Dr. I. A. Magnus using a powerful Quartz–Xenon monochromator.[18] Wave-bands between 390 mμ and 410 mμ were selected.

RESULTS

Urinary estimations

Table 1 gives the results found for the urinary levels of coproporphyrin, uroporphyrin, PBG and ALA after feeding rats on the five hexachlorobenzenes studied. Results for AIA and Sedormid-feeding are given for comparison. In each case the dose of chlorinated benzene was increased gradually until a level was reached which produced high porphyrin excretion yet with very few fatalities. These doses were, therefore, close to the lethal dose and the animals were killed when the porphyrin excretion appeared to have reached a plateau level.

In rats treated with chlorinated benzenes urinary coproporphyrin excretion became markedly raised (Table 1); this was most apparent with 1,3,4-trichlorobenzene and 1,4-dichlorobenzene. This rise usually appeared after a dose of 250–500 mg/kg and

TABLE 1. MEAN PEAK VALUES OF URINARY PORPHYRINS AND PORPHYRIN PRECURSORS FOLLOWING TREATMENT OF MALE RATS WITH THE MAXIMUM DOSES TRIED OF EACH CHLORINATED BENZENE; VALUES FOR ALLYLISOPROPYLACETAMIDE AND SEDORMID ARE GIVEN FOR COMPARISON

No. of rats	Compound	Solvent	Max. dose (mg/kg)	Days on max. dose	Coproporphyrin (μg/day)	Uroporphyrin (μg/day)	PBG (μg/day)	ALA (μ/gday)	ALA/PBG
	Controls*	C			4·3–6·8	0·1–0·3	2·5–6·5	38·7–51·6	
3	1-Cl.B.	P	1140	5	30·50	1·40	26·70	56·40	2·98
3	1,2-Cl.B.	P	455	15	43·10	2·01	26·65	11·32	2·11
3	1,4-Cl.B.	P	770	5	61·80	10·99	1328·10	437·41	0·36
3	1,2,3-Cl.B.	C	785	7	36·59	0·72	57·38	36·26	1·01
3	1,2,4-Cl.B.	C	730	15	58·31	2·73	179·00	145·70	1·06
3	1,2,3,4-Cl.B.	P	660	10	28·96	3·80	520·63	315·78	0·61
6	1,2,4,5-Cl.B.	C	905	5	4·10	0·22	6·50	18·08	6·69
3	AIA	Pr.g	250	10	17·35	5·00	863·67	571·45	0·71
2	AIA	Pr.g	500	6	43·07	16·61	2539·61	2378·68	0·96
4	Sedormid	Pr.g	250	10	8·32	3·60	851·56	444·99	0·52

Cl.B = chlorobenzene; P = liquid paraffin; C = 1% cellofas; Pr.g = propylene glycol; AIA = allylisopropylacetamide; PBG = porphobilinogen; ALA = δ-aminolaevulic acid.

* Mean max. and min. of 5 rats during 41 days

was the first apparent sign of the intoxication. All of the coproporphyrin excreted in the urine was identified as coproporphyrin III by means of paper chromatography; no other porphyrin intermediates were found. Alone of the six chlorinated benzenes tried, 1,2,4,5-tetrachlorobenzene had no effect on urinary porphyrin excretion. It was administered suspended in cellofas and the negative result may be due to poor absorption.

In contrast to the results found for urinary coproporphyrin excretion, the chlorinated benzenes produced only a small (though definite) rise in the excretion of urinary uroporphyrins; 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1,2,3,4-tetrachlorobenzene were the most effective chlorobenzenes in this respect (Table 1). These relatively small increases in urinary uroporphyrin excretion contrast with the results found with animals fed AIA or sedormid where there is a marked rise in uroporphyrin excretion.

Increases in urinary PBG excretion were not apparent as early as the increases in coproporphyrin and uroporphyrin and higher doses of the chlorinated benzenes were necessary to produce a significant effect (500–800 mg/kg). This was again in contrast to the AIA and sedormid groups where the urinary excretion of PBG rose very quickly to a high value (Table 1). The most noticeable effect on PBG excretion after ingestion of chlorinated benzenes was found with 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and 1,2,3,4-tetrachlorobenzene. Figure 1 shows the urinary excretion pattern obtained for coproporphyrin, ALA and PBG after feeding 1,2,3,4-tetrachlorobenzene.

Urinary ALA excretion was also increased after feeding chlorinated benzenes, most noticeable effects being found with those compounds mentioned previously in connection with PBG excretion. As was the case with PBG, the increases found in the urinary excretion of ALA after feeding chlorinated benzenes were in general far less than those obtained after ingestion of AIA or sedormid.

The urinary excretion of porphyrins and porphyrin precursors was found to be dependent upon the solvent used for administration of the solid chlorobenzenes. The urinary excretion was greater when the drugs were given dissolved in liquid paraffin or in propylene glycol than when they were administered as suspensions in cellofas (Table 2).

Liver estimations

Changes in the activity of ALA-dehydrase in liver tissue were followed after feeding rats on 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene and, for comparison, after feeding with AIA or sedormid. In the animals fed 1,2,4-trichlorobenzene there was a small rise in the liver enzyme activity (value found 1·25 μmoles PBG/g liver compared with 1·1 μmoles found for normal untreated male rats); for 1,2,3,4-tetrachlorobenzene the corresponding value was 1·43 μmoles PBG/g liver. These values were less than the values found after feeding AIA or sedormid (respective values 1·93 and 2·69 μmoles PBG/g liver).

Administration of chlorobenzenes was found to have no significant effect on the level of liver catalase (Table 3) in contrast to the marked decreases found in catalase activity after dosing with AIA or sedormid. Significantly decreased activities of liver catalase following administration of chlorinated benzenes were found only when histological examination showed severe liver damage with large areas of necrosis consequent on feeding toxic levels of these substances, especially the mono- and *o*-dichlorobenzenes (Table 3).

Following the administration of chlorinated benzenes there was a general rise in liver uroporphyrin and, in animals displaying marked porphyrinuria, there were increases in liver coproporphyrin and protoporphyrin. PBG was detected in liver tissue only in the terminal stages of some animals which were markedly porphyric. These results are given in Table 3.

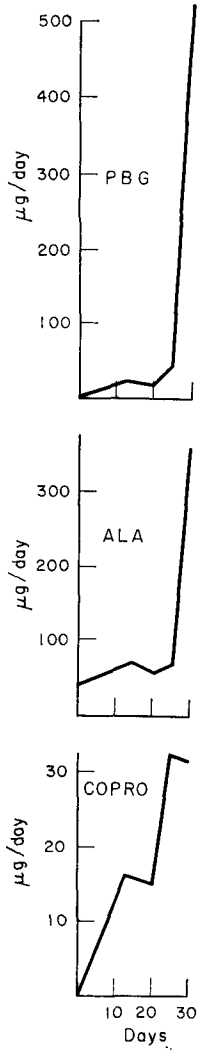

FIG. 1. Mean urinary excretion of coproporphyrin (Copro), δ-aminolaevulic acid (ALA) and porphobilinogen (PBG) of rats dosed 1,2,3,4-tetrachlorobenzene daily for 30 days (20 days with 400 mg/kg, the following 10 days with 660 mg/kg).

No change in the hepatic glutathione content was found following the administration of 1,4-dichlorobenzene, hexachlorobenzene, AIA or sedormid. A slight reduction in liver glutathione content was obtained after feeding 500 mg/kg 1,2,3,4-tetrachlorobenzene and a more definite reduction was found with a similar dose of 1,2,4-trichlorobenzene; these decreases were found 2 hr after administration of the the poison.

TABLE 2. EFFECT OF SOLVENT (C = 1% CELLOFAS; P = LIQUID PARAFFIN) ON INCREASE OF URINARY PORPHYRINS AND PORPHYRIN PRECURSORS IN RATS INTOXICATED WITH 1,4-DICHLOROBENZENE AND 1,2,3,4-TETRACHLOROBENZENE

No. of rats	Compound	Max. dose (mg/kg)	Days on max. dose	Solvent	Coproporphyrin (μg/day)	Uroporphyrin (μg/day)	PBG (μg/day)	ALA (μg/day)	ALA/PBG
3	1,4-Cl.B.	850	5	C	14·74	0·83	7·55	49·13	6·12
3	1,4-Cl.B.	770	5	P	61·88	10·99	1328·10	437·41	0·36
3	1,2,3,4-Cl.B.	685	10	C	15·87	Trace	22·78	11·40	2·02
3	1,2,3,4-Cl.B.	660	10	P	28·96	3·80	520·63	315·78	0·61

Cl.B. = chlorobenzene; PBG = porphobilinogen; ALA = δ-aminolaevulic acid.

TABLE 3. PORPHYRINS, PORPHOBILINOGEN AND CATALASE ACTIVITY IN LIVERS OF RATS TREATED WITH CHLORINATED BENZENES, ALLYLISOPROPYLACETAMIDE OR SEDORMID

No. of rats	Compound	Max. dose (mg/kg)	Days on max. dose	Coproporphyrin (μg/100 g)	Protoporphyrin (μg/100 g)	Uroporphyrin (μg/100 g, uncorrected)	PBG	Catalase (m.eq./mg wet wt.)*
	Controls*†			4·5	9·7	1·3	—	0·85
2	1-Cl.B.	1400	5	Trace	13·00	6·35	—	0·502
2	1,2-Cl.B.	450	15	10·05	34·80	14·40	—	0·364
2	1,4-Cl.B.	770	5	5·07	60·50	60·35	—	0·880
2	1,2,3-Cl.B.	780	7	2·65	3·55	20·00	—	0·857
5	1,2,4-Cl.B	500	10	42·56	55·60	52·70	++	0·747
3	1,2,3,4-Cl.B.	660	10	35·04	56·57	41·32	+	0·772
2	1,2,4,5-Cl.B.	850	5	6·30	9·90	2·15	—	0·838
3	AIA	250	10	17·42	19·10	13·08	++	0·320
1	AIA	500	6	73·60	114·50	18·49	++	0·140
2	Sedormid	250	10	9·29	12·91	17·07	++	0·220

* See Methods section. Cl.B. = chlorobenzene; AIA = allylisopropylacetamide; PBG = porphobilinogen.
† Mean of 2 rats.

The excretion of porphyrins and porphyrin precursors in rats receiving 500 mg/kg 1,2,4-trichlorobenzene was decreased to almost normal levels following daily intraperitoneal injection of 400 mg/kg reduced glutathione (Fig. 2). The decrease occurred within a few days even though the administration of trichlorobenzene was not stopped; after a few doses of glutathione, the animals appeared in better condition and began to increase in weight.

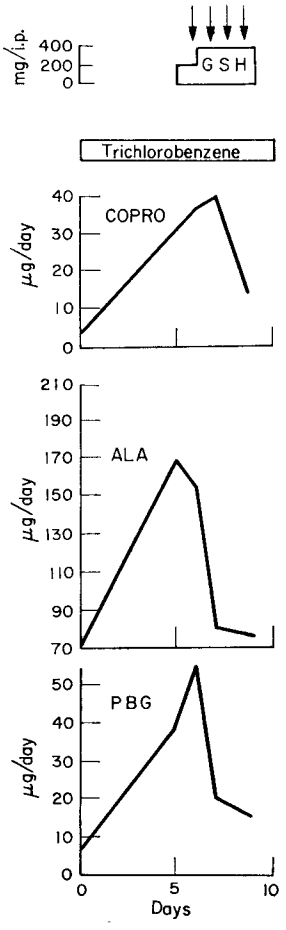

FIG. 2. Effect of intraperitoneally injected reduced glutathione (GSH) on the mean urinary excretion by three rats of coproporphyrin (Copro), δ-aminolaevulic acid (ALA) and porphobilinogen (PBG) whilst they received a daily oral dose of 1,2,4-trichlorobenzene (500 mg/kg).

The pyridine nucleotide contents of liver samples taken from rats fed 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, AIA and sedormid were estimated by Dr. T. F. Slater. The results are shown in Table 4. It can be seen that the feeding of chlorinated benzenes leads to an overall decrease in the liver pyridine nucleotide contents and that the ratios NAD–$NADH_2$ and NADP–$NADPH_2$ are changed to some extent.

Clinical and pathological results

The animals receiving chlorinated benzenes showed loss of weight and appetite. A profound constipation was not observed. Evidence of paralysis in the form of left-sided hemiparesis was only obtained in one rat treated with 1,4-dichlorobenzene. A common picture in the terminal stages of high porphyric rats was extreme weakness, ataxia, clonic contractions and, rarely, slight tremor. With exception of the animals

TABLE 4. PYRIDINE NUCLEOTIDE CONTENT OF RATS' LIVER AFTER TREATMENT WITH 1,2,4-TRICHLOROBENZENE, 1,2,3,4-TETRACHLOROBENZENE, ALLYLISOPROPYLACETAMIDE OR SEDORMID

No. of rats	Compound	Dose (mg/kg)	NAD (μg/g)	NADP (μg/g)	$NAD/NADH_2$	$NADP/NADPH_2$
	Controls		440	330	4·3	6·6
3	1,2,4-Cl.B.	500	303	335	5·6	5·7
2	1,2,3,4-Cl. B.	640	409	265	5·8	6·5
3	AIA	250	428	339	4·5	5·8
1	AIA	500	380	225	3·0	8·3
5	Sedormid	250	480	370	4·7	9·8

Cl. B. = chlorobenzene; AIA = allylisopropylacetamide.

treated with mono-chlorobenzene there was no decrease in haemoglobin content. Hepatomegaly was common in most porphyric rats. No or only slight liver-fluorescence could be detected under ultraviolet light, but a definite increase was noted after treatment with dilute iodine solution. Histologic examination revealed severe liver damage with intense necrosis and fatty change over large areas in animals treated with monochlorobenzene, 1,2-dichlorobenzene and 1,2,4-trichlorobenzene in high doses. The other chlorinated benzenes produced degeneration of individual liver cells without actual necrosis or small areas showing focal necroses in the central, midzonal and periportal regions.

The animals treated with 1,2,3,4-tetrachlorobenzene showed hair loss in the form of depilated spots covering smaller or larger parts of the skin without other lesions, particularly without any sign of light sensitivity or itching. The process was reversible, the hair grew again after dosing was discontinued. Histological examination of the skin revealed characteristic keratotic plugging of the follicles. As a result of this follicular hyperkeratosis there was atrophy and disintegration of the follicles and sebaceous glands. No infiltrate was present round the follicles. These alterations are probably due to stasis and pressure caused by the hyperkeratotic plugs within the follicle. Light sensitivity tests, carried out by irradiation of rats treated with mono-chlorobenzene, *o*-dichlorobenzene and AIA failed to produce any skin lesion.

DISCUSSION

Interest in the chemically induced porphyrias started with the recognition of the deleterious effects of Sedormid and closely related substances on patients with latent porphyria. The similar properties possessed by *allyl*-derivatives were discovered soon afterwards and the structural requirements for porphyria-induction have been investigated by and defined by Goldberg and Rimington.[19] A new class of compounds

capable of producing hepatic porphyria has been revealed by the discovery of the role of hexachlorobenzene in an outbreak of human cutaneous porphyria in Turkey.[1–3] The present work shows that continued ingestion by rats of the other chlorinated benzenes (with the exception of pentachlorobenzene) also leads to a hepatic type of porphyria characterised by much increased levels of porphyrins and porphyrin-precursors in the liver and excreta. The first sign of intoxication was a rise in urinary coproporphyrin III; rises in urinary PBG and ALA excretion were a later effect. The most active chlorobenzenes for inducing porphyria were those with two chlorine atoms in a *para-* position to one another (1:4-dichlorobenzene, 1:2:4-trichlorobenzene, 1:2:3:4-tetrachlorobenzene).

The porphyrin disturbances induced by these chlorinated benzenes showed differences not only from the earlier studies with hexachlorobenzene[4,5] (in that the uroporphyrin excretion was low) but also with AIA- and Sedormid-induced porphyria[19] (in that the rise in porphyrin precursors was a late event and high doses of chlorobenzenes were needed for this purpose). A further distinction between the effects produced by AIA and Sedormid compared with the chlorinated benzenes studied here was that the latter group of substances did not produce a significant decrease in liver catalase activity. Occasional decreases were observed but in these cases liver necrosis was appreciable. However, there were also similarities with the effects produced by AIA and Sedormid: PBG excretion increased more rapidly than ALA excretion so that the ALA/PBG ratio decreased as the toxicity of the chlorobenzenes became apparent. Also, liver ALA-dehydrase was raised after ingestion of the chlorinated benzenes but not to so great an extent as was found with AIA and Sedormid[14]; the most effective chlorobenzene in this respect was the 1:2:3:4-tetrachlorobenzene.

Ingestion of chlorobenzenes usually resulted in a relatively large rise in liver uroporphyrin content and, in animals severely affected by the drugs, there were also rises in liver copro- and protoporphyrins. Examination of fresh liver sections under ultraviolet light, however, showed little or no fluorescence. Fluorescence increased after treating the sections with dilute iodine indicating that a part of the liver porphyrin fraction was present as the non-fluorescent porphyrinogens.

Although it has been reported[20,21] that liver glutathione is decreased after feeding halogenated benzenes (a decrease which was related to the urinary elimination of mercapturic acids) only 1:2:4-trichlorobenzene of the substances tried here produced appreciable decrease in liver glutathione. This decrease occurred early (2 hr) after feeding the chlorobenzene and was to a large extent prevented if the animal was adequately fed. An interesting and important finding was, however, that the effects of trichlorobenzene on porphyrin metabolism could be largely prevented by concomitant administration of reduced glutathione.

Investigation of the detoxification of chlorinated benzenes shows that they are in part metabolised in animals to phenols, catechols and mercapturic acids.[20,22,23] In rabbits, as the number of chlorine atoms increases, mercapturic acid formation decreases and the higher polychlorinated benzenes are excreted as phenols.[24] Recently, it has been shown that glutathione can participate in liver mercapturic acid formation and Booth, Boyland and Sims[25] have shown the presence of a specific enzyme which couples glutathione with a halogenated aromatic substance with halogen elimation. A further point of relevance in this connection is that the toxicity of the chlorinated

benzenes (in terms of LD50) has been related to the subsequent utilisation of sulphur-compounds for mercapturic acid formation.[26] The mono- and *ortho*-dichlorobenzenes are the most toxic in this respect; *para*-dichloro-, and trichlorobenzene have only a slight effect on liver; the more highly chlorinated benzenes only produce liver damage in high doses and when fed for a long time. One possible explanation of this relative nontoxicity of the higher chlorobenzenes is that they are absorbed only poorly and inefficiently from the intestine and are only slowly metabolised. In fact, hexachlorobenzene seems to be virtually metabolically inert. Despite these indications of an interaction between chlorobenzenes and liver glutathione, however, only in the case of the 1:2:4-trichlorobenzene was a decrease of glutathione found. This would seem to indicate that the mechanisms producing porphyrin derangements are different from those leading to liver necrosis.

Finally, it is worth noting in connection with the cutaneous porphyria produced by hexachlorobenzene, that 1:2:3:4-tetrachlorobenzene produced in rats cutaneous lesions in the form of extended depilated areas. Skin histology revealed follicular hyperkeratosis similar to that seen in acneiform eruptions produced in humans and animals by other chlorinated aromatic hydrocarbons.[27]

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