THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 53, No. 6

Printed in U.S.A.

FACTORS AFFECTING THE INDUCTION OF PORPHYRIA IN THE LABORATORY RAT. BIOCHEMICAL AND PHOTOBIOLOGICAL STUDIES USING DIETHYL 1,4-DIHYDRO-2,4,6-TRIMETHYL-PYRIDINE-3,5-DICARBOXYLATE (DDC) AS A PORPHYROGENIC AGENT*

I. A. MAGNUS, M.D., F.R.C.P., D. A. ROE, M.D., M.R.C.P.† AND
L. K. BHUTANI, M.D.‡

ABSTRACT

Porphyria induced by the fluorochrome, diethyl 1,4-dihydro-2,4,6-trimethyl pyridine-3,5-dicarboxylate (DDC) has been studied in laboratory rats and the factors determining the pattern of porphyrin excretion have been assessed. Definitive studies demonstrating specific photosensitivity of the skin in the porphyric rats have been carried out.

It has been established that the magnitude of porphyrin excretion as well as the type of porphyric pattern is dosage dependent. Increased fecal protoporphyrin output can occur without other evidence of porphyria at a low dosage level. As the drug dosage is increased, fecal and urinary coproporphyrin and finally urinary uroporphyrin values are additionally increased. These changes in porphyrin output have been explained on the current theory that DDC inhibits the incorporation of protoporphyrin into heme and the production of heme compounds so that by a positive feedback mechanism ALA synthetase is activated and excess urinary copro and uroporphyrin excretion occurs.

The collidine derivative was well tolerated by animals receiving a conventional rat diet *ad libitum*, but in animals on purified diets a state of chronic nutrient deprivation precipitated acute toxicity. Toxic symptoms, associated with DDC intake at a high dosage level, included constipation, icterus, oliguria and premature death.

The primary hepatic lesion of DDC-induced porphyria consisted in biliary hyperplasia with a surrounding mononuclear infiltrate. Discrete aggregation of protoporphyrin within the bile ducts and in the periductal inflammatory cells was localized by polarization and fluorescence microscopy. At autopsy the animals exhibiting toxic symptoms showed massive hepatic necrosis and renal tubular damage.

In 1959, Solomon and Figge (1) demonstrated that the fluorochrome, diethyl 1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (DDC) was a potent porphyrogenic agent. When this chemical was fed to mice, a disturbance of porphyrin metabolism resulted, which was characterized by a marked increase in hepatic proto and coproporphyrin as well as deposition of protoporphyrin and much smaller amounts of uroporphyrin between the renal cortex and pelvis. In the guinea pig oral DDC, given for seven days, produced high levels of urinary coproporphyrins, but only trace amounts of porphobilinogen. Later studies by these authors showed that the porphyrogenic effects of DDC were not shared by its oxidation products (2). These results have in general been confirmed by other workers, though there has been controversy about their interpretation. While De Matteis and Prior concluded that the experimental hepatic porphyria induced by DDC was similar to that induced by drugs of

The authors are indebted to Professor Lennart Krook and Dr. Bruce Wilkie of Cornell University for the histological studies, and to Dr. Francesco de Matteis of the Medical Research Council, Toxicology Branch, Carshalton, England for advice on the preparation and utilization of DDC.

Received June 10, 1969; accepted for publication July 17, 1969.

* From the Institute of Dermatology, St. John's Hospital for Diseases of the Skin, Homerton, London E.9, England and the Graduate School of Nutrition, Cornell University, Ithaca, New York.

† The initial phase of the work was conducted during the tenure of the Hazel Clifford Postgraduate Studentship, University of London, and completed with the support of a grant from the Nutrition Foundation #403.

‡ Financial assistance was obtained from the British Council and the Wellcome Trustees.

the Sedormid group (3), several investigators have shown that DDC and chemically related drugs cause a more persistent block in heme and heme compound formation, resulting in massive accumulation of protoporphyrin in the liver (4, 5).

The data presented in this communication pertain to studies of DDC-induced porphyria in the laboratory rat. The primary aim has been to evaluate whether the laboratory rat is a suitable experimental animal model for use in screening drugs or industrial chemicals for porphyrogenic properties. Relationships of dosage level to porphyria induction, the contribution of endogeneous porphyrins from the Harderian glands to porphyrin excretion and the association between porphyria and chemical toxicity have been studied.

Another objective has been to study the relation of porphyria induction to dietary deprivation. The epidemic of porphyria cutanea tarda in rural populations of Southeastern Turkey, due to ingestion of seed wheat treated with hexachlorobenzene, occurred at a time of relative famine (6). Numerous publications have stressed that the disease was most severe in growing children (7, 8, 9). We have therefore studied the effects of caloric restriction as well as protein deprivation on young rats receiving porphyrogenic agents. This research has been supplemented by photobiological studies as an index of the severity of the porphyria. Here we report the effects of DDC; results of a similar study with hexachlorobenzene will be reported later.

MATERIAL AND METHODS

Drug and drug dosage. DDC, obtained from Eastman Kodak, was recrystallized from hot ethanol and the yellow impurity removed by washing the crystals on a Buchner funnel with 3N HCl, followed by distilled water. The crystals were dried, under reduced pressure, in a dessicator. The pure compound was suspended in Arachis oil at a concentration of 80 mg/ml, and given by gastric intubation with a syringe attached to a blunt-ended metal needle. Three dosage levels of DDC were used, viz: 80 mg, 200 mg and 400 mg/kg body weight. Arachis oil alone was given to control animals in comparable amounts to that given to the experimental animals.

Animals. Male Wistar albino rats were used in these studies. The starting weights of rats on the conventional rat chow diets varied between 200 and 350 gm and those on the purified diets between 105 and 168 gms. The animals were individually housed in wire bottom "holding" cages and transferred for porphyrin determinations to special cages that permitted complete separation of urine and feces. Metabolic collections were made over 48 hour periods.

Diets. The rats were fed a pelleted rat chow (MRC 41B) ad libitum except in the experiments designed to show the effect of protein depletion on the induction of porphyria (Table IA and IB). In these nutritional studies the animals were divided into two groups and pair fed purified diets so that the intake of the rats on the "high protein"

TABLE IA

Composition of pelleted rat chow MRC 41B

	%
Wheat	47.0
Sussex ground oats	40.0
Fish meal (66% protein)	8.0
Dried skim milk	3.0
Dried yeast (unextracted)	1.0
Salt	1.0
	100.0

A vitamin supplement is added at 2.5 lbs/ton which supplies oil and water soluble vitamins in adequate quantities to meet rat requirements. Molasses (5%) is added to the dry diet to bind ingredients.

TABLE IB

Composition of purified rat diets

	Low protein	High protein
Casein	8.0	25.0
Corn oil	12.6	5.0
Corn starch	55.0	55.0
Choline	0.3	0.3
Salt mix[a]	4.0	4.0
Fat soluble vitamins[b]	1.0	1.0
B vitamin mix[c]	2.0	2.0
Cellulose (Alphacel)	17.1	7.7
	100.0%	100.0%

[a] Salt mix H.M.W., Nutritional Biochemicals, see ref. 10.

[b] Fat soluble vitamin mix: Vitamin A acetate 62.0 mg., vitamin D 0.9 mg., α-tocopherol 1000.0 mg., corn oil to 200 gm.

[c] B vitamin mix: Thiamine 0.4 gm., pyridoxine 0.4 gm., riboflavin 0.8 gm., Ca pantothenate 4.0 gm., niacin 4.0 gm., inositol 20.0 gm., menadione 1.0 gm., folic acid 0.2 gm., biotin/B_{12} (3 mg/20 mg) 10.0 gm., cerelose to 2 kg.

diet was restricted to that of the animals on the "low protein" diet. The diets of the two animal groups were made isocaloric by substitution of corn oil in the diet of the protein depleted animals.

Surgical procedures. Removal of the Harderian glands in selected rats anesthetized with Nembutal was carried out by dissection under Wood's light. Combined wet weights of the glands were measured immediately after removal. The rats were returned to the holding cages after this procedure until complete recovery had taken place as determined by food consumption and normal activity.

Biochemical studies. Extraction and isolation of urinary and fecal porphyrins was carried out using Rimington's method (11). Harderian gland porphyrins were extracted according to the method of Schwartz (12). Spectrophotometric measurement of individual porphyrins was performed using either the Hilger Uvispek or the Beckman DU Spectrophotometer.

Qualitative determination of urinary bilirubin was made using a commercially prepared diazo reagent. (Bili-Test Tablets—a Scientific Product, Evanston, Illinois).

Photobiological studies. Irradiations were made using a high intensity water-prism monochromator with a 2 K.W. high pressure quartz xenon arc source (13). Wave length calibration of the monochromator was carried out with a low power, high pressure polykymatic quartz mercury vapor arc before each test irradiation. For monochromatic irradiations at wave length 420 nm or below, a W.G.3 Schott absorption filter was inserted in the optical path of the monochromator to remove any scattered U.V. below 320 nm. For longer wave lengths in the visible spectrum a G.G. 475 Schott filter was used.

Measurements of the irradiance at the exit slit of the monochromator were made with a calibrated low vacuum thermopile (Hilger F.T. 17) with SiO_2 window. All measurements should, however, be taken as approximate and relative rather than absolute.

The irradiations of the animals were made on the shaved flank skin on which the sites to be irradiated had been marked out in such a way to avoid overlap of reactions. The animals were held firmly in a position which brought the marked area into close contact with the exit slit. No skin site was irradiated more than once.

The first few animals were irradiated with monochromatic light at 400 nm to assess (a) if the animals would react at all to this wave length, and (b) to observe the sequence of reactions taking place at the irradiated sites.

In the preliminary experiments it was found that animals became photosensitive to 400 nm after 7 to 12 doses of DDC and would react to 2700 mW. sec/cm^2 or less of this monochromatic light. In later experiments therefore all animals were routinely irradiated after 10 doses of DDC using 400, 350, 380, 420, 500, 600 and 700 nm wave bands and multiple exposures with varying dosages of light in order to determine the "minimal dose for response" (MDR) at each of these wave lengths. A minimum of 5 animals were used for each wave length. Five normal rats, who were not given DDC, served as controls and were irradiated in a like manner to determine the MDR. These animals were the same in which porphyrin excretion values were obtained.

Early reactions were read by "blueing" the skin with an intravenous or intraperitoneal injection of 3 ml of Coomassie Blue solution. These injections were made immediately after the end of an exposure and the site of irradiation inspected peridocially up to 48 hours. After 48 hours reactions were assessed visually without use of Coomassie Blue.

The absorption spectrum of DDC was determined with a recording spectrophotometer (Unicam SP 800) (Fig. 1).

Pathology techniques. At the end of each experiment the animals were sacrificed by decapitation. The hair, skin and teeth as well as the viscera were examined under Wood's light for evidence of gross porphyrin fluorescence. Histological sections were cut from paraffin blocks of the livers, kidneys and lungs. Sections were stained with hematoxylin and eosin for routine light microscopy. Polarization optical examination and fluorescence microscopy of unstained sections was carried out.

RESULTS

Drug dosage level. Alterations in porphyrin excretion, induced by DDC, varied with the dose (Table II). Elevation of fecal protoporphyrin excretion followed initiation of DDC administration, even at the lowest dosage employed, viz., 80 mg/kg body weight. In the rats fed ad libitum on rat chow, this was accompanied by a moderate increase in fecal coproporphyrin excretion and small increases in the excretion of urinary copro and uroporphyrin. At the higher dosage level, *viz.*, 400 mg/kg body weight, the same pattern of change in porphyrin excretion occurred, but the level of porphyrins in the feces and in the urine was higher. Substantially different gains in fecal protoporphyrin excretion were found from rat to rat.

Duration of DDC administration. Chronic administration of DDC caused a decrease in fecal porphyrin and an increase in urinary porphyrin excretion in some of the experimental animals. Analysis of the data showed that these trends were not significant for the rat groups as a whole and no inverse relationship between fecal and urinary porphyrin levels was established (Table III).

Effect of Harderian gland ablation. Two rats were subjected to Harderian gland excision

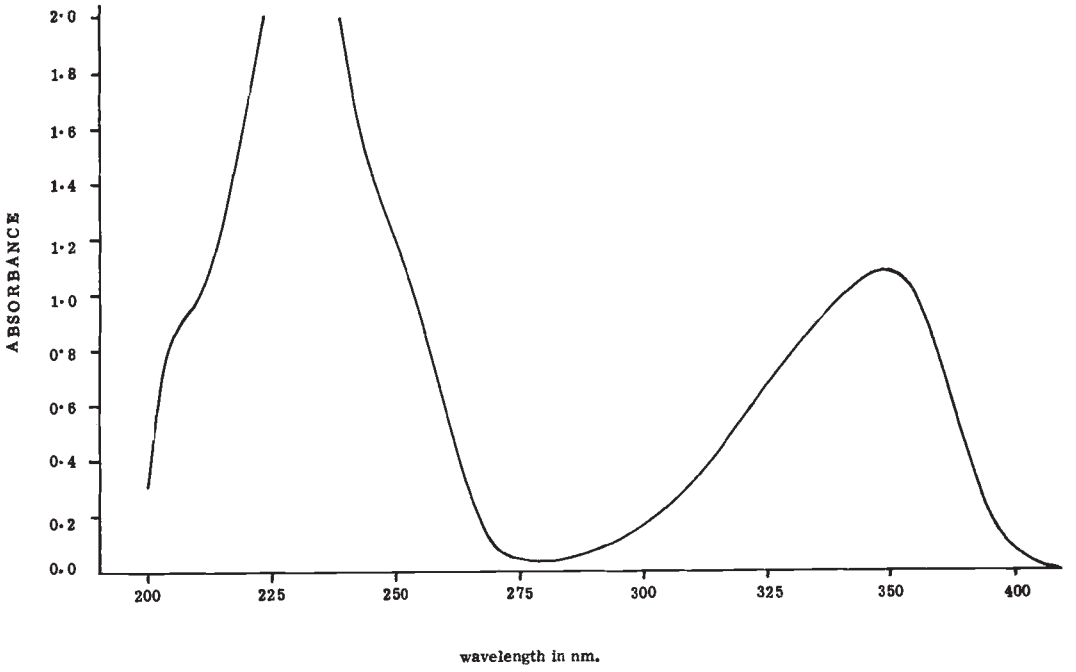

FIG. 1. Absorption spectrum of DDC. The porphyrogenic agent was dissolved in ethanol to give a 1.87×10^{-10} molar solution. Measurements were made on the Unicam SP 800.

TABLE II

Changes in porphyrin excretion in rats following DDC administration

(Data collected from rats fed conventional rat diet ad libitum)

Group	No. rats	Wt. gm. Mean ± S.D.	DDC dosage mg/Kg	Urinary porphyrins μg/24 hrs. (mean values)		Fecal porphyrins μg/gm. dry wt. (mean values)	
				Copro	Uro	Copro	Proto
A	3	233 ± 14	0	1.93	0.11	1.60	27.03
			80	5.10	0.36	12.07	84.59
B	11	314 ± 40	0	9.50	0.51	8.75	38.94
			400	32.85	5.03	37.89	328.98

Post-DDC porphyrin values were obtained from composite data of all metabolic collections made from 10–20 days after drug initiation.

Statistical evaluation of group A was precluded by limited data available.

In group B very highly significant post-DDC increases in fecal protoporphyrin ($t = 4.04$ $P < 0.005$), fecal coproporphyrin ($t = 5.47$ $P < 0.001$), urinary coproporphyrin ($t = 4.69$ $P < 0.001$) and urinary uroporphyrin ($t = 4.97$ $P < 0.001$).

prior to DDC administration. After recovery from this procedure they showed marked reduction in fecal protoporphyrin output (Table IV). When DDC was given to these animals at a level of 80 mg/kg body weight, one showed only moderate increases in fecal protoporphyrin output, while the other displayed the massive increase in protoporphyrin excretion, which was characteristic of other animals fed the drug at this dosage. A possible explanation for the difference was found at autopsy, for under Wood's light the Harderian gland ablation was complete in the former rat, whilst in the latter there were remnants *in situ*.

Dietary effects. The effect of DDC dosage level on porphyrin excretion was clearer in

TABLE III

Porphyrin excretion of rats on chronic DDC intake

Mean and range of values in longitudinal study of 14 animals on conventional diet receiving DDC at a dosage level of 400 mg/Kg. The data refer to rats used in the photobiological studies (Table 7).
n = number of metabolic collections.

Duration of DDC intake (days)	*n*	Urinary porphyrins μg/24 hrs.		Fecal porphyrins μg/gm dry wt.	
		Copro	Uro	Copro	Proto
0	14	9.50 (0.63–35.20)	0.62 (0.22–1.58)	8.44 (2.72–13.63)	37.56 (16.50–63.19)
3	4	34.33 (4.60–99.50)	2.12 (0.54–4.95)	68.19 (35.47–94.60)	640.33 (377.60–853.00)
8	4	40.38 (15.40–54.80)	4.23 (1.70–7.07)	42.71 (21.74–64.58)	307.09 (128.06–484.80)
11	2	21.12 (11.19–31.04)	1.35 (0.16–2.54)	37.34 (36.79–37.88)	417.40 (359.60–475.20)
13	4	34.83 (28.70–51.50)	6.87 (4.70–10.03)	40.26 (27.13–55.20)	387.69 (213.56–539.90)
15	2	19.44 (16.62–22.25)	3.02 (2.79–3.25)	64.15 (61.20–67.10)	374.89 (257.38–492.40)
17	2	40.81 (23.07–58.54)	6.11 (3.74–8.48)	18.85 (16.64–21.05)	180.54 (142.48–218.60)
18	4	39.46 (20.44–67.50)	5.49 (1.97–12.10)	49.63 (38.50–60.70)	373.13 (190.40–468.30)
27	3	22.56 (19.14–25.35)	1.32 (0.81–1.61)	72.12 (43.97–114.20)	348.00 (283.70–457.40)

rats fed purified diets (Table V). On a daily intake of the drug of 80 mg/kg the only significant change in porphyrin excretion, even after 10 days, was an elevation of fecal protoporphyrin excretion. The gains in fecal protoporphyrin excretion were not significantly higher in the rats on a high protein diet at this dosage level of the drug. At 200 mg/kg the massive gains in fecal protoporphyrin content were similar in rats on high and low protein intake. Changes in fecal coproporphyrin output were not significant ($P > 0.05$). A small but significant rise in urinary coproporphyrin output occurred at this dose level, but the differences in the urinary porphyrin output between the two diet groups was not statistically significant. Given DDC at a level of 400 mg/kg body weight, elevation in fecal copro and protoporphyrin excretion was similar in both groups, but urinary coproporphyrin and uroporphyrin excretion was significantly higher in the rats fed a low protein diet.

Photosensitivity. The initial experiments designed to study whether porphyria induced by DDC resulted in photosensitivity showed that most animals with the lower doses of DDC and all animals with the higher dosage schedules reacted to monochromatic irradiation at 400 nm. The degree of photosensitivity was more marked with the higher dosages of DDC.

An evanescent, inconstant erythema appearing immediately after the end of an exposure and lasting for 3–5 minutes was noticed in the more photosensitive animals. The erythematous

area did not "blue" after an intravenous injection of "Coomassie Blue."

The immediate erythema was followed by a period of latency varying in duration between 1 to 4 hours, which in turn was followed by an erythema with or without edema. This was better visualized as "blueing" after parenteral administration of "Coomassie Blue." After 18–24 hours the positive reaction appeared as "blueing" or "blanching" (pale center with blueing at the periphery after an injection of Coomassie Blue) and lasted for 36–48 hours (Fig. 2a, b, c). Occasional lesions showed ecchymoses at the site of irradiation. No bulla or flare was seen. In the less sensitive animals these changes were followed by a brownish pigmentation, scaling and atrophy, while in the more sensitive animals after higher doses of light these progressed to necrosis and ulceration which healed with scar formation.

The action spectroscopic studies showed that none of the animals, normal or porphyric, reacted at 600 and 700 nm even with the highest doses of light employed in these experiments. At 500 nm, 4 out of 5 animals; at 380 nm, 3 out of 5; and at 350 nm, 2 out of 5 animals reacted with blueing and at times edema. In contrast to these results, all the animals responded with blueing with or without edema to monochromatic light at 400 nm and 420 nm. Some of the rats also showed immediate erythema. The MDR at 400 nm was less than at 420 nm. None of the control animals showed any reaction with the highest doses of light employed (Table VII).

Evidence of DDC toxicity. Animals receiving the conventional rat diet ad libitum tolerated the drug at the highest dosage level, viz., 400 mg/Kg body weight, without evidence of acute or chronic toxicity, except photosensitivity associated with the porphyria. However the groups of animals which were pair fed on purified diets displayed marked signs of acute toxicity. This toxicity was much more severe in the rats on the high protein intake and led to premature death. All rats showed weight loss, and in the rats on the high protein diet, signs of combined hepatic and renal damage. The rats on the 25% casein diet exhibited rapid weight loss, jaundice, constipation and diminishing urinary output within three days of receiving DDC at the dosage level 400 mg/Kg body weight and animals died rapidly after these symptoms were apparent (Table VI). Positive correlation was obtained between weight gain prior to DDC and weight loss after drug administration for animals on both high and low protein intake (Coefficient of Rank Correlation R = 0.730). There was no evidence of neurotoxicity.

Pathology of DDC intoxication. At autopsy, rats receiving DDC at all dosage levels showed macroscopic porphyrin fluorescence of the stomach and intestine. In addition, rats receiving the drug at a dosage level of 400 mg/kg showed intense porphyrin fluorescence localized to hemorrhages within the lungs.

The toxic animals had pathological signs of massive liver necrosis and renal tubular necrosis with casts blocking the distal renal tubules (Fig. 3a, b, c). All rats receiving DDC, regardless of the dosage level, showed a primary

TABLE IV

The effect of Harderian gland ablation on fecal porphyrin content

Rat #	Status of Harderian gland	DDC dosage		Fecal porphyrins μg/gm dry wt.	
		Level, mg/Kg	Days	Copro	Proto
A	Intact	0	0	0.59	36.69
	Ablation	0	0	1.08	2.48
	Ablation	80	3	7.98	96.88
B	Intact	0	0	0.05	18.09
	Ablation[a]	0	0	3.30	4.96
	Ablation	80	3	45.30	565.72
C	Intact	0	0	1.62	34.15
	Intact	80	3	12.81	475.67
D	Intact	0	0	1.62	17.37
	Intact	80	3	33.76	311.77

Rat #	Total wet wt. of glands mg.	Harderian gland porphyrins, μg/gm wet wt.			
		Copro[b]	Proto	Uro	Total
A	281.5	132.78	161.69	0.91	295.38
B	199.1	63.28	78.56	0.21	142.05

[a] Remnants of Harderian glands found in rat B at autopsy.

[b] Harderian coproporphyrin fraction may include tricarboxylic compounds.

TABLE V

The effect of DDC dosage level on the porphyrin excretion of rats pair-fed on purified diets
(Values represent mean increases or decreases in urinary and fecal porphyrins)

Group	Wt. gm Mean ± S.D.	# Rats	DDC dosage mg/Kg	Duration DDC admin. (days)	Urinary porphyrins μg/24 hrs.		Fecal porphyrins μg/gm dry wt.	
					Copro	Uro	Copro	Proto
L.P.	148 ± 9	4	80	10	−1.99	−0.06	−1.84	+57.96
H.P.	156 ± 17	4	80	10	+0.39	−0.27	+2.47	+148.81
L.P.	187 ± 9	4	200	3	+8.80	−0.71	+15.48	+393.21
H.P.	200 ± 22	4	200	3	+5.95	+1.00	+23.49	+493.61
L.P.	185 ± 27	8	400	3	+32.98	+2.64	+22.31	+289.71
H.P.	202 ± 10	8	400	3	+8.35	−4.96	+13.49	+283.64

Statistical evaluation of post DDC increases in porphyrin excretion

a) *Dose dependent changes (both diet groups)*

Significant or very highly significant increases in fecal protoporphyrin in rats on all dosage levels, viz: 80 mg/Kg $t = 3.52$ $P < 0.01$, 200 mg/Kg $t = 2.35$ $P < 0.05$, 400 mg/Kg $t = 7.08$ $P < 0.001$.

Significant increases in fecal coproporphyrin in rats on 400 mg/Kg dose only $t = 2.82$ $P < 0.05$.

Highly significant increases in urinary coproporphyrin excretion in rats receiving drug at higher dosage levels, viz: 200 mg/Kg $t = 5.23$ $P < 0.01$, 400 mg/Kg $t = 4.86$ $P < 0.005$ (L.P.) and $t = 5.52$ $P < 0.001$ (H.P.).

Significant increases in urinary uroporphyrin excretion in rats on 400 mg/Kg dose only $t = 2.82$ $P < 0.05$.

b) *Low protein vs. high protein fed animals*

Very highly significant differences in urinary porphyrin excretion in rats on 400 mg/Kg dose, viz: copro $t = 4.17$ $P < 0.001$, uro $t = 4.79$ $P < 0.001$.

hepatic lesion consisting in hyperplasia of the bile ducts and ductules (Fig. 4a, b). Surrounding the bile ducts there was a mononuclear infiltrate consisting in histiocytes and lymphocytes. Localized deposition of protoporphyrin, in crystal form, was present within the bile ducts and within and between the cells of the mononuclear infiltrate.* Hyaline degeneration of the cells of the hepatic parenchyma and necrosis amounting to complete loss of the normal liver structure was present in the rats with severe acute toxicity. Massive hemorrhages were present within the liver substances and damage to the endothelial lining of the sinusoids was obvious.

DISCUSSION

It appears that in evaluating the effect of a chemical compound on hepatic porphyrin metabolism factors imposed by the experimental conditions have to be considered. Our studies with DDC have shown that overt porphyrinuria is associated only with high dosage levels of the compound. With lower dosages of DDC there was greater variability in the pattern of porphyrin excretion and the only uniform finding in rats maintained under differing experimental conditions was an increased fecal protoporphyrin output. It also appeared that if a low dose rate of DDC was maintained over a long period of time that there was a diminution

* The identification of the crystalline deposits in the liver as protoporphyrin has been established in weanling pigs treated with DDC. In these animals the primary hepatic lesion is identical with that found in the rats but birefringent crystalline aggregates were also found in the bile. These aggregates had the solubility and absorption characteristics of protoporphyrin.

In a rat study carried out subsequent to the preparation of this paper, it was found that hepatic protoporphyrin levels were elevated after 3 days treatment with DDC at a dosage level of 400 mg/kg. Liver copro and uroporphyrin was not significantly raised in this experiment.

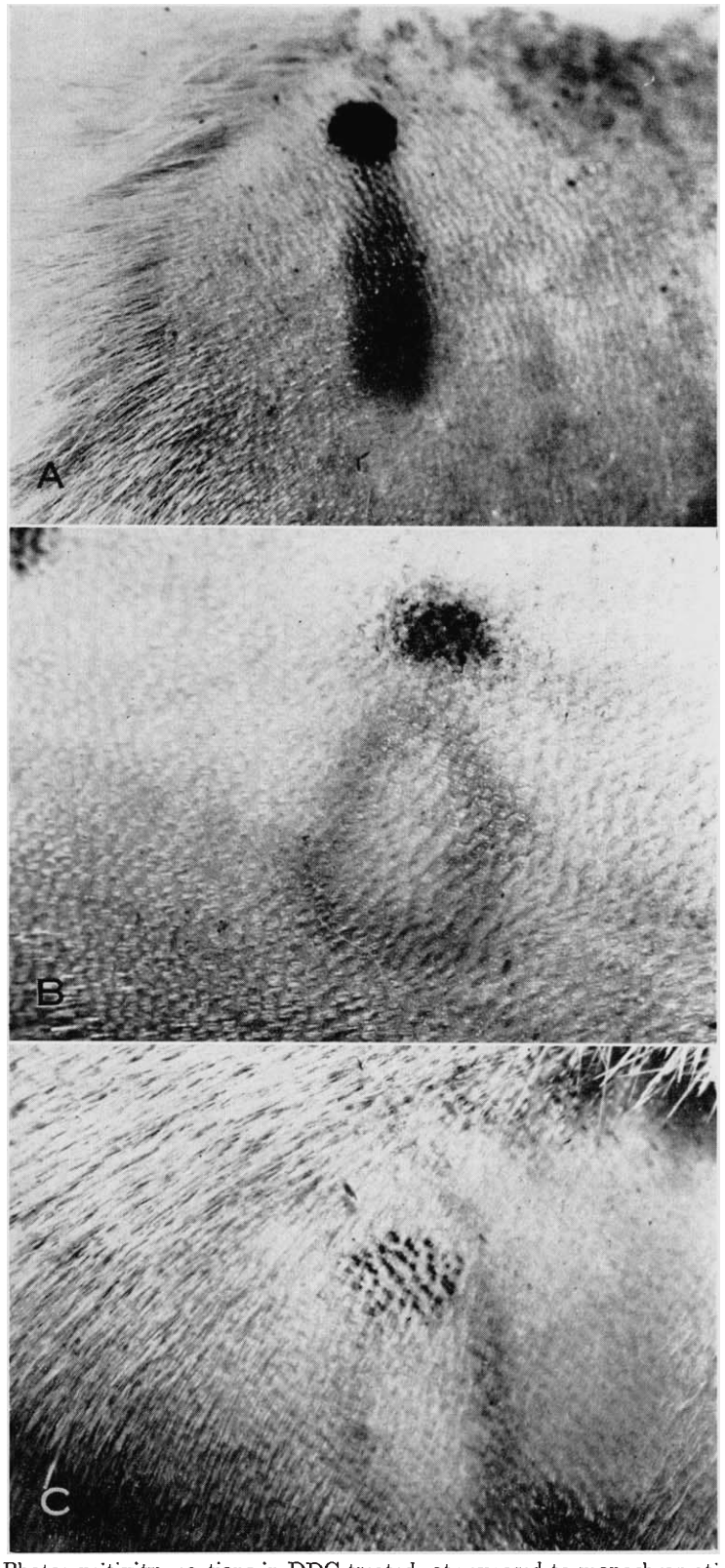

FIG. 2. Photosensitivity reactions in DDC-treated rats exposed to monochromatic light at 400 nm. Visualization of delayed cutaneous responses following i.v. injection of Coomassie Blue. a) Erythema immediately after test exposure. b) Delayed erythema after 24 hours, showing peripheral "blueing" and central blanching reaction. c) Edema reaction lasting from 24–72 hours after irradiation. In Fig. 2 a) and b) ecchymosis can be seen at the site of irradiation.

TABLE VI

Drug toxicity in rats receiving purified diets plus DDC at a dosage level of 400 mg/g

Rat	Diet		Pre-DDC wt. gain	Post-DDC 3 day wt. loss	Duration of DDC → death	Premortal evidence of toxicity		
	Type	Duration				Constipation[a]	Icterus[b]	Oliguria[c]
					(days)			
1	L.P.	2/12	67	−18	13 (S)	−	−	−
2	L.P.	2/12	66	−17	40 (S)	−	−	−
3	L.P.	2/12	77	−13	40 (S)	−	−	−
4	L.P.	2/12	61	−14	40 (S)	−	−	−
9	L.P.	3/12	−9	+7	3 (S)	−	−	+
10	L.P.	3/12	74	−4	3 (S)	−	−	−
11	L.P.	3/12	76	−15	3 (S)	+	−	−
12	L.P.	3/12	69	−9	3 (S)	−	−	−
19	H.P.	2/12	99	−25	5	−	+	+
20	H.P.	2/12	92	−14	4	−	+	−
21	H.P.	2/12	96	−18	4	−	+	+
22	H.P.	2/12	61	−12	13 (S)	−	−	−
27	H.P.	3/12	121	−21	3 (S)	+	+	−
28	H.P.	3/12	96	−28	3 (S)	+	+	+
29	H.P.	3/12	80	−16	3 (S)	+	+	−
30	H.P.	3/12	85	−15	3	+	+	+

[a] Constipation refers to absence of post-DDC fecal output.
[b] Icterus denotes positive test for urinary bile pigments.
[c] Oliguria defined as urinary output <4.0 ml/24 hrs.
S = sacrifice by decapitation.

of porphyrin output. Reduction of porphyrogenic potency on chronic feeding may be related to a drug-induced detoxification mechanism. Thus, to ascribe a particular pattern of porphyrin excretion to a specific porphyrogenic agent may be misleading, unless this is related to the level and duration of intake.

The toxic properties of DDC primarily effect the liver and there has been no evidence of neurotoxicity such as may be associated with the administration of porphyrogenic compounds like the halogenated benzenes (14, 15).

It was shown by Onisawa and Labbe that after DDC administration, the livers of mice were firm on palpation and dark red in color (16). When DDC was given to the guinea pigs, Granick and Urata demonstrated the localization of protoporphyrin within the hepatic parenchyma by fluorescence microscopy (17). These authors also showed that there were red brown granules within the liver cells which to polarized light appeared as trichite crosses. It was suggested that these birefringent masses consisted of crystalline needles of protoporphyrin in a rosette-like arrangement. Chronic poisoning with DDC was shown to result in cirrhosis. In the rat, toxicity to DDC is manifested by anorexia, sudden loss of weight, constipation, oliguria and jaundice. These symptoms are similar to those described by de Matteis and Rimington in griseofulvin toxicity (18). Both in the case of griseofulvin and with DDC, there is a premortal decrease in the urinary excretion of pyrrole compounds. The signs and symptoms of toxicity can be explained from the histological findings, though it is somewhat difficult to isolate the toxic effects of DDC on the liver from those due to deposition of porphyrin. The primary hepatic lesion due to the collidine derivative is deposition of protoporphyrin crystals in and around the bile ducts and ductules, and a periductular foreign body reaction. The question arises whether secondary necrosis of the hepatic parenchyma is due to the collidine derivative is deposition of tasis from duct blockage by crystalline porphyrin. Although Rimington (19) has stated that diffuse hepatic poisons destroy the concentrating power of the liver cell, so that uptake of

porphyrins is prevented and renal excretion consequently occurs, this generalization is not applicable without qualification to the case of DDC. There has been no evidence of an inverse relationship between the excretion of fecal and urinary porphyrins. Rats, in the premortal state, excrete no porphyrins from the large intestine. This however, is due to absolute constipation and when the contents of the large intestine are extracted, they are found to contain massive amounts of protoporphyrin and smaller amounts of coproporphyrin.

Decreased urinary excretion of porphyrins prior to death can be explained on the basis of the renal lesions which include necrosis of the distal convoluted tubules as well as the collecting tubules which are blocked by casts. It may be assumed that renal tubular passage of porphyrins is depressed by the kidney pathology. At present it is not possible to decide whether the renal lesions are due to direct DDC toxicity or are secondary to the hepatic changes. Porphyrin excretion can only be a measure of porphyrin synthesis if the kidney is intact and intestinal function within physiological limits.

Tolerance for DDC is greatest in rats receiving a conventional rat chow diet ad libitum. These animals received the collidine derivative at a dosage level of 400 mg/kg body weight without showing evidence of toxicity. Among rats on purified diets, the rats which were protein depleted tolerated the chemical much better than those on a high protein intake. The simplest explanation of this rests on the fact that the high protein group were maintained under conditions of relative caloric deprivation in relation to their growth requirements, brought about by the technique of pair-feeding. The premise that hepato-toxicity of DDC is influenced by growth rate is supported by the positive correlation between weight gain prior to DDC and weight loss afterwards (see Table VI). In a recent study, it was shown that if a drug metabolite is more toxic than the parent compound, then protein depletion exercises a protective effect because drug metabolism in the liver is depressed (20). This principle might also explain the effects observed with DDC.

Normal coprophagy in the rat (21) limits utilization of changes in fecal porphyrin output as a measure of altered porphyrin metabolism.

TABLE VII

*Relationship between wave length and minimal dose for cutaneous response (MDR) in porphyric and control rats exposed to monochromatic light**

Wave length nm.	Porphyric group		Control group	
	# rats	MDR mW.sec./cm²	# rats	MDR mW. sec./cm²
350	2	1350	5	>2700
	3	>2700		
380	3	675	5	>2700
	2	>2700		
400	4	330	5	>2700
	2	675		
	1	1350		
	3	2700		
420	2	675	5	>2700
	2	1350		
	1	2700		
500	4	2700	5	>2700
	1	>2700		
600	5	>2700	5	>2700
700	5	>2700	5	>2700

* Values given in this table refer to rats fed MRC 41B diet ad libitum. Porphyric animals had received DDC (400 mg/kg) for 10 days.

In the present limited studies it has also been demonstrated that the porphyrin secretion of the Harderian gland, which is ingested by the rat, contributes significantly to the fecal protoporphyrins, and this has indeed been suggested by earlier studies (22).

It is difficult to classify the type of experimental porphyria induced by DDC. Apparently the type of porphyria produced is dependent on the dosage level of the drug. At the higher dosage level the pattern of porphyrin excretion suggests the changes associated with so-called symptomatic cutaneous porphyria (porphyria cutanea tarda). However on the lower dosage levels, viz., 80 mg/Kg, the picture shows similarity to the congenital erythropoietic protoporphyria occurring in man. Thus, there was massively increased fecal protoporphyrin excretion and a liver pathology which has been associated

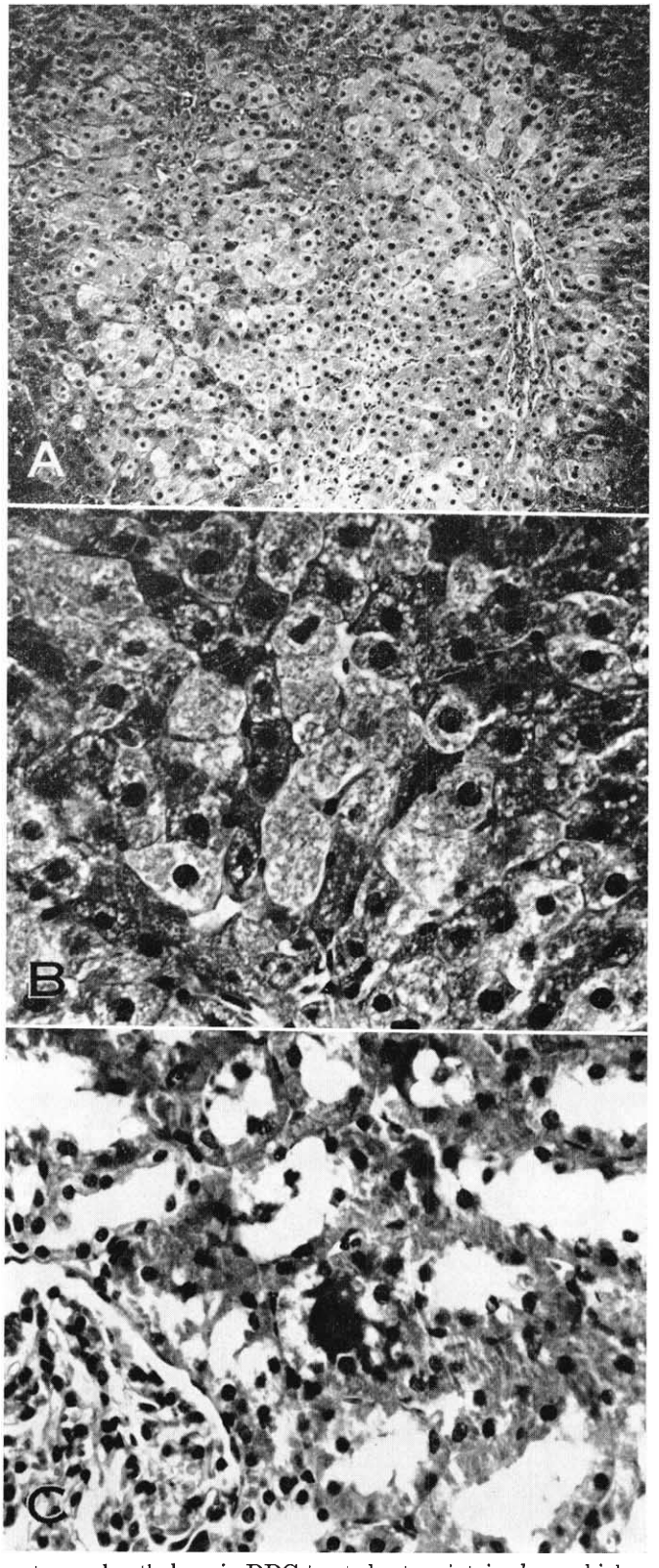

FIG. 3. Hepato-renal pathology in DDC-treated rat maintained on a high protein purified diet. Animal sacrificed after 3 days of DDC administration (400 mg/kg). a) Low power view of liver showing hepato-cellular necrosis, maximal around portal tracts (H and E × 103). b) High power view of liver showing individual cell necrosis, pyknotic nuclei and hyaline bodies in the cytoplasm (H and E × 387). c) High power view of kidney showing necrosis of tubular epithelium and cast in situ. (H and E × 387).

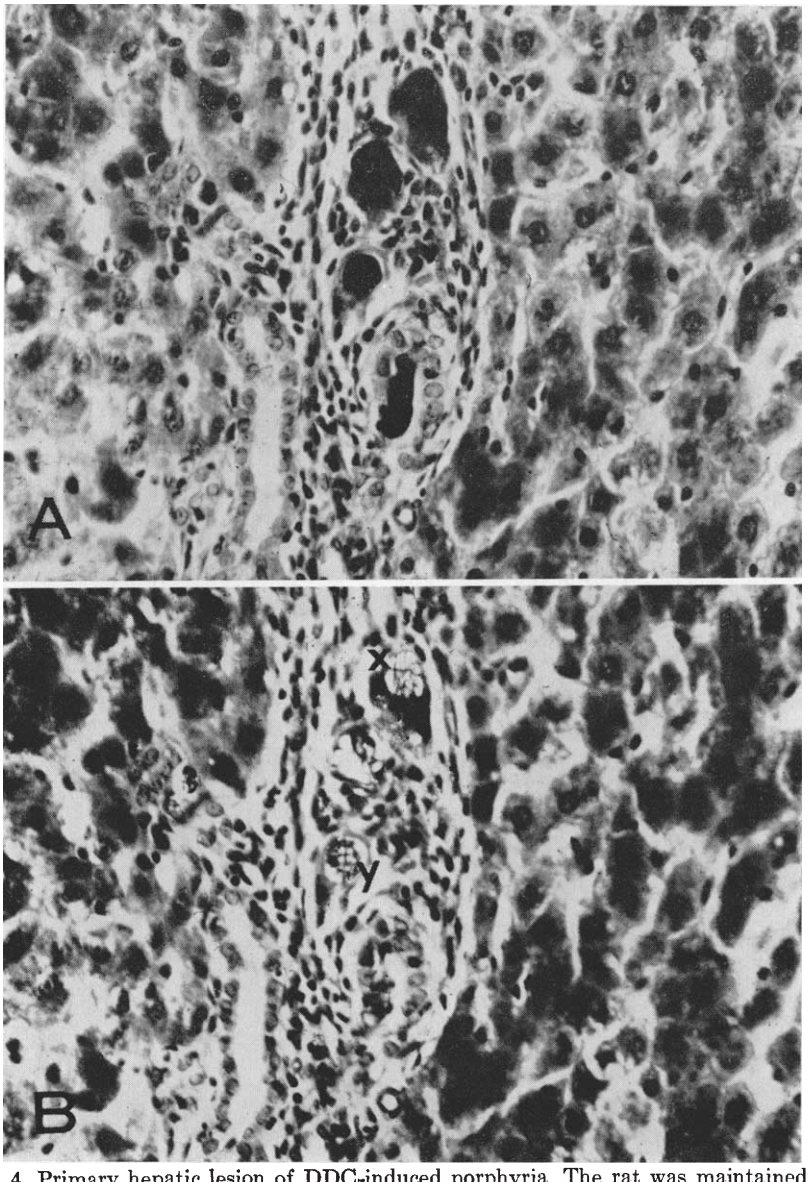

FIG. 4. Primary hepatic lesion of DDC-induced porphyria. The rat was maintained on a high protein purified diet and received DDC at a dose of 80 mg/kg for 53 days. a) High power view of liver showing biliary hyperplasia and surrounding stromal infiltrate of histiocytes and lymphocytes. Large pigment masses are situated within and between the bile ductules. Both hepatocellular necrosis and regeneration can be seen in the liver parenchyma (H and E × 300). b) High power view of liver under polarized light showing crystalline deposits within a bile duct (X) and Maltese cross figures in the surrounding stroma (Y) (H and E × 300). The birefringent material corresponds in location with the pigment masses which showed characteristic porphyrin fluorescence.

with this form of porphyria (23). In the absence of erythrocyte porphyrin assays in our studies, the localization of porphyrin fluorescence to hemorrhages within the lungs may lend some support to this explanation. Furthermore, de Matteis noted raised erythrocyte porphyrins after DDC administration to rats and mice (24, 25).

It is known that DDC inhibits the incorporation of protoporphyrin into heme (16). If it is assumed that heme is the normal repressor of ALA synthetase activity (26), then with dimin-

ished formation of this end-product, ALA synthetase would be activated and this would result in the over-production of abnormal liver porphyrins. Presumably with liver damage, these porphyrins would be reabsorbed into the circulation and eventually excreted in the urine. This would explain the different patterns of porphyrin excretion occurring in the rat groups which were studied.

The present study, to our knowledge, is the first successful attempt at delineating the action spectrum for photosensitivity in porphyric animals. These investigations showed that the most reactive wave length in these animals was 400 nm. The reactivity at 420, 380 and 500 nm was less marked than at 400 nm. The results are in conformity with the results of action spectrum in human porphyric patients (27, 28, 29, 30), and correspond closely with the absorption spectrum for porphyrins, thereby satisfying the requisites of the Grotthus-Draper Law. Again this action spectrum is quite unlike the spectrum for normal sunburn.

Because a few animals reacted to monochromatic light at 350 and 380 nm, the suggestion has been retained that the photosensitivity could in part be due to the direct effect of DDC in the skin. However in view of the characteristic evaluation of the skin changes, this seems unlikely. These experimental studies lend further support to the evidence already presented in humans (27, 30) concerning the role of porphyrins in the production of photosensitivity and would tend to negate the contention of Blum (31) and Clare (32) that cutaneous porphyrins play no part in the photosensitivity of porphyria.

The pattern of the response observed on irradiating the skin, with minor differences, is similar to that seen in human subjects with porphyria (30). Immediate erythema, delayed erythema with or without edema, scaling, atrophy, ecchymoses, have all been observed in human patients. The differences in time-course of these reactions and the absence of bullous lesions and flare, may possibly be because of species differences and differences in the structure of skin. Again, the biphasic response observed does not seem to be peculiar to photosensitivity in porphyric rats, since similar response has been reported in rats in inflammation secondary to heat or U.V. irradiation (33, 34). The inconsistency of the immediate erythema is possibly related to the degree of photosensitivity since only the more photosensitive animals showed this.

The obvious cause for these photosensitive reactions would seem to be the presence of increased amounts of porphyrins in the skin. While no attempt was made to determine the porphyrin content of skin in this study, that this may be elevated has been shown by Pathak and Burnett (35) in porphyric patients and hexachlorobenzene-induced porphyric rats.

The morphology of the photosensitivity reactions has been interpreted by Stratigos and Magnus (36) and more recently by Hunter, Bhutani and Magnus (37). It was shown that the initial erythema is due to vaso-congestion of the horizontal plexus of dermal capillaries, but with the onset of edema, the microscopic view of the congested vessels is obscured and this is associated with pallor of the skin. In severe reactions, thrombi develop and the overlying edema is absorbed very slowly. Thus pallor of the reaction site after Coomassie Blue injection is due to failure of the dye to enter the vessels which are congested and thrombosed.

REFERENCES

1. Solomon, H. M. and Figge, F. H. J.: Disturbance in porphyrin metabolism caused by feeding diethyl 1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate. Proc. Soc. Exp. Biol. Med., *100:* 583, 1959.
2. Solomon, H. M. and Figge, F. H. J.: Porphyrin metabolism in mice following quantitative administration of collidine compounds and oxidation products. Proc. Soc. Exp. Biol. Med., *105:* 484, 1960.
3. De Matteis, F. and Prior, B. E.: Experimental hepatic porphyria caused by feeding 3,5-diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine. Comparison with Sedormid porphyria. Biochem. J., *83:* 1, 1962.
4. Haeger-Aronsen, B.: Porphyria induced in the rabbit by diethyl 1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate. II. Catalase activity and concentration of green porphyrins in the liver and a comparison with Apronal-induced porphyria. Acta Pharmacol. Toxicol., Kbh. *19:* 156, 1962.
5. Wada, O., Yano, Y., Urata, G. and Nakao, K.: Behavior of hepatic microsomal cytochromes after treatment of mice with drugs known to disturb porphyrin metabolism in liver. Biochem. Pharmacol., *17:* 595, 1968.
6. Cam, C.: Cutaneous porphyria related to in-

toxication. Dirim (Istanbul in Turkish), *34:* 11, 1959.
7. Dogramaci, I.: An outbreak of toxic porphyria in southeastern Turkey. Turkish J. Pediat., *3:* 57, 1961 (Reprinted in English).
8. Schmid, R.: Cutaneous porphyria in Turkey. New Eng. J. Med., *263:* 397, 1960.
9. Cetingil, A. I. and Ozen, M. A.: Toxic porphyria. Blood, *16:* 1002, 1960.
10. Hubbell, R. B., Mendel, L. B. and Wakeman, A. J.: A new salt mixture for use in experimental diets. J. Nutrit., *14:* 273, 1937.
11. Rimington, C.: Quantitative determination of porphobilinogen and porphyrins in urine and feces. Assoc. Clin. Path. Broadsheet (n.s.) No. 36, 1961.
12. Schwartz, S., Berg, M. H., Bossenmaier, I. and Dinsmore, H.: Determination of porphyrins in biological materials, p. 221, *Methods of Biochemical Analysis* Vol. 8. Ed., Glick, D., Interscience Publ. Inc., New York, London, 1960.
13. Magnus, I. A., Porter, A. D., McCree, K. J., Moreland, J. D. and Wright, W. D.: A monochromator. An apparatus for the investigation of the responses of skin to ultraviolet, visible and near infra-red radiation. Brit. J. Derm., *71:* 261, 1959.
14. De Matteis, F., Prior, B. and Rimington, C.: Nervous and biochemical disturbances following hexachlorobenzene intoxication. Nature (London), *191:* 363, 1961.
15. Rimington, C. and Ziegler, G.: Experimental porphyria in rats induced by chlorinated benzenes. Biochem. Pharmacol., *12:* 1387, 1963.
16. Onisawa, J. and Labbe, R. F.: Effects of diethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate on the metabolism of porphyrins and iron. J. Biol. Chem., *238:* №2, 724, 1963.
17. Granick, S. and Urata, G.: Increase in activity of δ-aminolevulinic acid synthetase in liver mitochondria induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine. J. Biol. Chem., *238:* №2, 821, 1963.
18. De Matteis, F. and Rimington, C.: Disturbance of porphyrin metabolism caused by griseofulvin in mice. Brit. J. Derm., *75:* 91, 1963.
19. Rimington, C.: Pp. 325–331, *Biliary Secretion of Porphyrins and Hepatogenous Photosensitization in the Biliary System.* Ed., Taylor, W., F. A. Davis Co., Philadelphia, 1965.
20. Weatherholtz, W. M., Campbell, T. C. and Webb, R. E. Effect of dietary protein levels on the toxicity and metabolism of heptachlor. J. Nutrit., *98:* 90, 1969.
21. Barnes, R. H.: Nutritional implications of coprophagy. Nutrit. Rev. *20:* №10, 289, 1962.
22. Goldberg, A. and Rimington, C.: chap. 7, p. 155, *Diseases of Porphyrin Metabolism.* Charles C. Thomas, Springfield, Ill., 1962.
23. Cripps, D. J. and Scheuer, P. J.: Hepatobiliary changes in erythropoietic protoporphyria. Arch. Path., *80:* 500, 1965.
24. De Matteis, F.: Unpublished observations.
25. De Matteis, F.: Disturbances in liver porphyrin metabolism caused by drugs. Pharm. Rev., 19: №4, 523, 1967.
26. Kappas, A. and Granick, S.: Steroid induction of porphyrin synthesis in liver cell culture. II. The effects of heme, uridine diphosphate glucuronic acid and inhibitors of nucleic acid and protein synthesis on the induction process. J. Biol. Chem., *243:* №2, 346, 1968.
27. Magnus, I. A., Porter, A. D. and Rimington, C.: The action spectrum for skin lesions in porphyria cutanea tarda. Lancet, *1:* 912, 1959.
28. Magnus, I. A., Jarrett, A., Prankerd, T. A. and Rimington, C.: Erythropoietic protoporphyria. A new porphyria syndrome with solar urticaria due to protoporphyrinemia. Lancet *2:* 448, 1961.
29. Holti, G., Magnus, I. A. and Rimington, C.: Erythropoietic protoporphyria in sisters. Brit. J. Derm., *75:* 225, 1963.
30. Rimington, C., Magnus, I. A., Ryan, E. A. and Cripps, D. J.: Porphyria and photosensitivity. Quart. J. Med., *36:* 29, 1967.
31. Blum, H. F.: Pp. 225–235, *Photodynamic Action and Diseases Caused by Light.* Haffner Publ. Co., Inc., New York (Reprint of 1941 edition).
32. Clare, N. T.: P. 693, *Radiation Biology,* Vol. 3. Hollaender, A., New York, 1956.
33. Wilhelm, D. L. and Mason, B.: Vascular permeability changes in inflammation. The role of endogenous permeability factors in mild thermal injury. Brit. J. Exp. Path., *41:* 487, 1960.
34. Logan, G. and Wilhelm, D. L.: Vascular permeability changes in inflammation. I. The role of endogenous permeability factors in ultraviolet injury. Brit. J. Exp. Path. *47:* 300, 1966.
35. Pathak, M. A. and Burnett, J. W.: The porphyrin content of skin. J. Invest. Derm., *43:* 119, 1964.
36. Stratigos, J. D. and Magnus, I. A.: Photosensitivity by demethylchlortetracycline and sulphanilamide. Brit. J. Derm., *80:* 391, 1968.
37. Hunter, J. A. A., Bhutani, L. K. and Magnus, I. A.: Chlorpromazine photosensitivity in mice: Its action spectrum and the effect of antiinflammatory agents. Brit. J. Derm. In press.