Excretion of Coproporphyrin in Rats Developing Acute Massive Hepatic Necrosis.* (17432)

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Massive hepatic necrosis in rats maintained on diets low in sulfur containing amino acids has been well described by Himsworth and Glynn.[1] This is a rapidly fatal disease which appears suddenly in a hitherto healthy animal. The lesion has been produced by feeding diets deficient in cystine and methionine where all of the nitrogen was supplied by other amino acids.[2] Hock and Fink[3] and Himsworth and Glynn[1] regularly observed hepatic necrosis on diets containing yeast as a sole source of protein. Recently Gyorgy[4] has described the regular production of this lesion when a certain type of yeast was employed as the protein source. The suddenness with which illness and death appear in such animals has been well documented[5,6] and no satisfactory biochemical means of predicting the onset of this lesion have been described. The present study was undertaken to determine whether or not an increase in the urinary excretion of coproporphyrin might herald the development of massive hepatic necrosis.

The urinary excretion of coproporphyrin in liver disease has been discussed in recent reviews.[7,8] An increase of urinary coproporphyrin is frequently regarded as a sensitive index of liver functional impairment. Clinical applications have been recently described by Watson and his associates[9] with particular reference to the distribution of the type I and type III isomer.

Glynn, Himsworth and Neuberger[2] stated that in the course of their studies on massive hepatic necrosis, the urines from certain groups of rats had been examined for porphyrins. No significant increases were found; the actual values obtained were not presented. Dent and Rimington,[6] during their attempts to produce acute hepatic necrosis, observed the rapid development of porphyrinuria in rats fed a diet which included oxidized casein. Although the diets were devoid of methionine and contained very little cystine, the rats failed to develop acute necrosis of the liver. This study, however, left unanswered the question raised above, viz: whether a heightened excretion of coproporphyrin might precede the manifestations of massive liver necrosis.

Methods. The diet employed in this experiment was identical with those employed by Gyorgy.[4]† The composition of the diet was powdered yeast,‡ 18%, cornstarch, 79% and salt mixture, 3%. Each animal received 8 g of the mixture per day. Each daily portion was supplemented with 0.5 cc of peanut oil and 2 drops of cod liver oil. Daily, each rat received 1.0 cc of a solution containing thiamine chloride 20 γ, calcium pantothenate 100 γ, pyridoxine 20 γ and riboflavin 25 γ.

The rats were maintained in individual cages. Weights were recorded once each week.

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1 Himsworth, H. P., and Glynn, L. E., *Clin. Science*, 1944, **5**, 93.

2 Glynn, L. E., Himsworth, H. P., and Neuberger, A., *Brit. J. Exp. Path.*, 1945, **26**, 326.

3 Hock, A., and Fink, H., *Z. Physiol. Chem.*, 1943, **274**, 187.

4 Gyorgy, Paul, Liver Injury, Transactions of the Eighth Conference, Josiah Macy Jr., Foundation, New York, 1949, in press.

5 Himsworth, H. P., The Liver and Its Diseases, Oxford, 1947, Blackwell Scientific Publications.

6 Dent, C. E., and Rimington, C., *Biochem. J.*, 1947, **41**, 253.

7 Dobriner, K., and Rhoads, C. P., *Physiol. Rev.*, 1940, **20**, 416.

8 Watson, C. J., and Larson, E. A., *Physiol. Rev.*, 1947, **27**, 478.

9 Watson, C. J., Hawkinson, V., Capps, R. B., and Rappaport, E. M., *J. Clin. Invest.*, 1949, **28**, 621.

† The initial supply of yeast employed in these studies was made available to us by Dr. Gyorgy.

‡ "Kiln dried baking yeast," United Yeast Co., Ltd., 241 City Road, London E.C.I.

Food was supplied, in weighed amounts, every 2 days and water was allowed freely. Urine and feces collections were made for 48 hour periods at various intervals during the course of the study. During the collection period the rats were housed in individual plastic cages with glass rod bottoms. These cages were suspended over large glass funnels with 5 cm openings at the tapered end. Satisfactory separation of urine and feces, with little or no contamination, was accomplished by means of a specially devised glass tube attached to the lower end of the funnel. This successfully collected the urine at the side, where it drained into a tube, and permitted the feces to drop straight through into a separate container. The precaution of employing only glass and plastic in these devices was deemed necessary to prevent any contact of the urine with metal. We have noted the formation of metal porphyrin complexes during other studies when metal cages were employed, as have others also.[6] The collection periods were interrupted for 60 minutes at the end of the first 24 hours to permit feeding. An average of 3 to 5 cc of clear urine was obtained during each 24 hour period from rats weighing 40 to 90 g each. The sides of the funnels were washed down with 10 cc of water at the end of each collection period. The urine plus the washings were then refrigerated. The coproporphyrin analyses were carried out on the total 48 hour urine and fecal collections within 12 hours of the final collection time.

The estimation of the fecal coproporphyrin was conducted according to the method of Schwartz and Zagaria.[10] This method was slightly modified to permit its use in the analyses of the urine samples. During the analyses of both urine and feces, the initial 5% HCl samples were each diluted to 0.4% and extracted exhaustively with chloroform to remove protoporphyrin and deuteroporphyrin. The completeness of the separation of the coproporphyrin from these two porphyrins was checked by examining the chloroform washings under the ultra-violet light. The coproporphyrin was quantitated by readings in the fluorophotometer (Lumetron) using a solution of 1 μg of coproporphyrin per 1 cc as the standard reference.

Estimation of the coproporphyrin isomer content was performed by pooling the 1% HCl fractions from 3 urine or 3 fecal collections. Procedure B of the method described by Schwartz and his co-workers[11] was employed for the determination of the percentage of isomer I and III.

Results. Experimental observations were made on 21 rats whose initial weights ranged from 36 to 88 g. The data obtained is summarized in Table I. Fourten of the rats died spontaneously between the 30th and 125th day. Each of these animals exhibited the characteristic gross and microscopic findings of acute massive hepatic necrosis as described and illustrated by Himsworth.[5] Representative specimens are shown in Fig. 1 and 2. In most instances the rats were found dead; in a few instances the abrupt change from an apparently normal healthy status to a moribund one was observed in a matter of a few hours. Occasionally convulsions preceded death. No attempt was made to administer glucose solution, though hypoglycemia has not been excluded as a possible cause of the terminal convulsive seizures.

The remaining 7 animals were sacrificed after 41, 51 and 59 days as shown in Table I. In no instance in this group was it possible to detect gross or microscopic changes in the liver.

The data relating to the urinary and fecal coproporphyrin excretion is shown in Table I. Because of other experiments it was not possible to house the animals in the special collecting chambers all of the time; random samplings were therefore obtained. Control values for 48 hour collections of urine and feces in 6 rats maintained on a diet of fox chow just before the start of this experiment are shown in Table I. An additional 6 rats of similar weight and maintained on a similar control diet revealed urinary values of 4 to 20 γ of coproporphyrin and fecal values of

10 Schwartz, S., and Zagaria, R., Metallurgical Laboratory Report CH-3600, Chicago, 1946.

11 Schwartz, S., Hawkinson, V., Cohen, S., and Watson, C. J., *J.B.C.*, 1947, **168**, 133.

TABLE I. Urine and Fecal Coproporphyrin Excretion in μg per 48 hr in Rats Maintained on an 18% Yeast Diet.

Rat No.	Sex	Material studied	Control† values	Day of experiment 11	19	23	31	35	39	42	44	49	77	Day terminated	Initial wt (g)	Final wt (g)	Outcome	Hist. find. (liver)
52	F	U			7									30	40	42	D	Massive necrosis
		F			5													
55	M	U			8		14							38	42	60	D	”
		F			3		5											
53	M	U			15		17							41	36	48	D	”
		F			4		7											
54	M	U			4		12							41	36	53	D	”
		F			5		4											
56	M	U		22		11		19	6*					41	70	57	D	”
		F		9		2		3	2*									
43	M	U	16						19					44	73	78	D	”
		F	9						8									
63	M	U						25			9	6		58	58	71	D	”
		F						3			5	5						
44	M	U	16						25					59	66	82	D	”
		F	11						5									
41	M	U	15						13					59	71	80	D	”
		F	5						5									
60	M	U		21		19		22		12		15		59	60	86	D	”
		F		6		8		4		7		6						
65	F	U						19			12	23		62	63	75	D	”
		F						4			2	4						
40	M	U	18						20					74	74	86	D	”
		F	9						5									
45	M	U	18										16	125	78	88	D	”
		F	6						5				13					
42	M	U	10						18				17	79	88	90	D	”
		F	8						9				18					
50	M	U			18		13							41	40	65	S	Normal
		F			7		5											
51	M	U			5		13							41	40	44	S	”
		F			10		9											
57	M	U		34		19		20		17		13		51	65	69	S	”
		F		4		5		6		4		3						
58	M	U		18		11		18		15		15		51	60	58	S	”
		F		9		4		4		5		6						
59	M	U		22		23		14		10		13		51	63	66	S	”
		F		5		4		4		4		5						
64	M	U						14			16	23		59	62	74	S	”
		F						3			3	4						
61	M	U		24		8		13		19		6		81	60	69	S	”
		F		9		2		2		3		2						

U—Urine. F—Feces.
D—Spontaneous death. S—Sacrificed.
* 24 hr collection; animal died during second 24 hr period.
† Animals maintained on stock diet (Fox Chow).

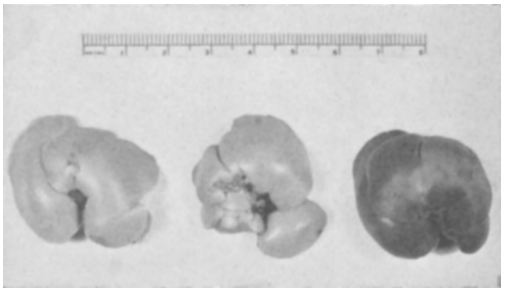

FIG. 1.
Gross appearance of rat liver showing massive hepatic necrosis as contrasted with unaffected experimental animal and a normal control, photographed after 4 hours fixation in formalin. The dark areas in the right hand specimen represent extensive areas of hemorrhagic necrosis (rat 56, death on 41st day). The center specimen (rat 51, sacrificed on 41st day) revealed no evidence of necrosis. The specimen to the left was taken from a normal animal of similar weight.

6 to 13 γ for 48 hour periods.

It is apparent from these data that no significant increases in the urinary excretion of coproporphyrin occurred in these animals. Rat 56 died suddenly during the second 24 hours of the collection period. The urinary and fecal values obtained during the first period (Table I) were not elevated. Estimation of the coproporphyrin isomer content was made in pooled samples of the material under study. Eighty to 90% of the coproporphyrin present behaved as the type III isomer by the differential precipitation "fluorescence quenching" technic.[11] A similar finding was noted in urine and fecal samples from the normal rats.

As has also been noted by others,[2,6] a few of the animals in this study exhibited traces of bilirubin in the urine one or two days before death but this was not common or constant. No increases in urobilinogen were observed; urinary amino acid excretion was not studied. A few specimens of urine were examined for the presence of homogentisic acid with negative results. The presence of this or a closely related substance in the urine of rats developing hepatic necrosis was observed by Glynn, Himsworth and Neuberger.[2] This occurred in rats fed an amino acid diet deficient in cystine and methionine. The question as to whether or not not renal damage might have prevented the excretion of pathological amounts of coproporphyrin may be raised. Histologically the kidneys of these animals appeared normal.

The excretion of excessive amounts of coproporphyrin III in the urine of humans is a

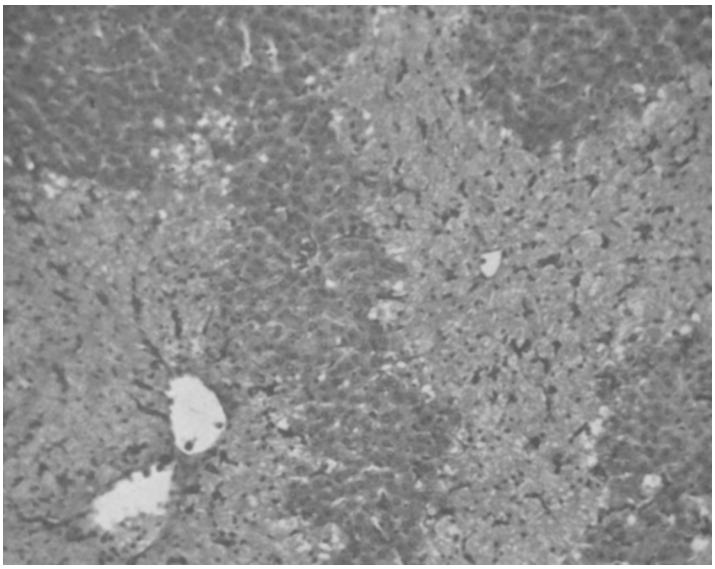

FIG. 2.
Photomicrograph, liver, rat 56, areas of necrosis in contrast with normal appearing liver cells, are shown in this section. In most sections, the areas of necrosis are more extensive. (H and E $\times$ 125).

frequent accompaniment of chemical or heavy metal toxicity, with or without evidence of hepatic functional impairment.[8,9] The failure to observe excesses in this experiment is in better agreement with the belief that the acute massive hepatic necrosis is not due to a chemical or poison. The histologic appearance of these livers suggests that the lesion develops very rapidly since there appears to be little evidence of antecedent damage as judged by infiltration or any attempt at a reparative process.

Summary. 1. The urinary and fecal excretion of coproporphyrin was studied at varying intervals in 21 young rats maintained on a diet containing 18% yeast as a source of protein.

2. Fourteen of the rats died suddenly between the 30th and the 125th day of acute massive hepatic necrosis. The gross and microscopic findings were identical with those described by Himsworth as characteristic of this lesion.

3. No significant increase in the excretion of coproporphyrin was observed during the course of these experiments. This is believed to minimize the likelihood of chemical or metal toxicity as the cause of the necrosis.

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Comparative Effect of Aureomycin and Chloromycetin on Psittacosis Infection in Chick Embryos.* (17433)

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Aureomycin[1] and chloromycetin[2,3] are antibiotics which have a similar spectrum of activity that includes many bacteria, rickettsias and viruses of the psittacosis-lymphogranuloma venereum group. With respect to psittacosis, Wong and Cox[4] found aureomycin to be highly effective against infection of embryonated hen's eggs with the 6BC strain, and Smadel and Jackson,[5] using the same strain, demonstrated similar beneficial effects from chloromycetin. The conditions of the experiments of these 2 groups of workers, however, were not entirely comparable. It seemed of interest, therefore, to make a direct comparison of the effects of these 2 agents under more nearly identical conditions. Such a comparison is reported in this paper.

Materials and methods. A uniform source of virus for the present experiments was obtained in the following manner: A stock yolk-sac suspension of the 6BC strain of psittacosis virus was injected into the yolk sac of a number of 7-day chick embryos which were then incubated at 35°C. On the 5th day, when deaths began to occur, the yolk sacs were harvested and pooled. An equal volume of broth was added and the mixture homogenized in a Waring blendor. The resulting 50% yolk sac suspension which, on culture, was found to be free of bacterial contamination, was distributed in sealed pyrex ampoules, quick-frozen and stored at −70°C. The LD_{50} of this suspension was about 10^{-8} throughout these experiments. Yolk sac smears of infected embryos stained by Macchiavello's technic showed numerous characteristic elementary bodies.

A fresh solution of crystalline aureomycin

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† Research Fellow in Virus Diseases, National Research Council.

1 Duggar, B. M., and others, *Ann. N. Y. Acad. Sci.*, 1948, **51**, 175.

2 Gottlieb, D., Bhattacharyya, P. K., Anderson, H. W., and Carter, H. E., *J. Bact.*, 1948, **55**, 409.

3 Smith, R. M., *et al.*, *J. Bact.*, 1948, **55**, 425.

4 Wong, S. C., and Cox, H. R., *Ann. N. Y. Acad. Sci.*, 1948, **51**, 290.

5 Smadel, J. E., and Jackson, E. B., *Science*, 1947, **106**, 418.