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DRUG-INDUCED PORPHYRIN BIOSYNTHESIS—II SIMPLE PROCEDURE FOR SCREENING DRUGS FOR PORPHYRIA-INDUCING ACTIVITY*

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Abstract—A series of drugs has been tested for porphyria-inducing activity in the intact chick embryo and in mice and the results compared with those previously obtained in chick embryo liver cells grown on cover slips. The results obtained in the intact chick embryo corresponded in the majority of cases with results obtained with the isolated liver cells. For this reason the intact chick embryo may be used as a rapid and simple procedure for screening drugs for porphyria-inducing activity. Although many of the drugs which induced porphyrin accumulation in the chick embryo liver appear to be inactive in mouse liver, evidence is presented that the results obtained with chick-embryo liver allow better prediction of results to be expected in porphyric patients than the results in mouse liver.

RECENTLY Granick[1] devised a procedure for demonstrating the porphyria-inducing activity of drugs. The method consists of adding drugs to a monolayer of chick-embryo liver cells grown on coverslips. The active drugs induce increased synthesis of δ-amino-levulinic acid synthetase resulting in the accumulation of porphyrins which are readily visualized with the fluorescence microscope. On the basis of results obtained with this procedure Granick[2] cautioned against the use of glutethimide, methsuximide, barbiturates, mephenytoin, griseofulvin and other drugs in patients and relatives of patients with porphyria. Watson[3] has suggested that many pharmaceuticals, especially sedatives and analgesics, should be screened by this procedure as latent cases of acute intermittent porphyria are more prevalent than has hitherto been realized.

We have observed[4, 5] that several drugs which are highly active in the liver-cell culture system are inactive when given orally to guinea pigs. It therefore appeared that on the basis of the test *in vitro* several valuable drugs might be unnecessarily excluded from use. It was thus of interest to determine whether the liver cells grown as a monolayer culture responded in the same way to porphyria-inducing drugs as the liver of the intact chick embryo. Talman *et al.*[6] determined the porphyria-inducing activity of a variety of drugs by injecting them into the yolk sac of 8-day-old chick embryos and measuring the porphyrin content of the allantoic fluid. In this paper we describe a simplified procedure for determining porphyria-inducing activity in the intact chick embryo by measuring porphyrin accumulation in the liver. In further studies the activity of drugs was determined in mice in order to compare the responsiveness of avian and mouse liver to porphyria-inducing drugs.

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EXPERIMENTAL

Fertilized eggs used were of a White Leghorn strain obtained from the University of Alberta farm and stored at 10° for no longer than 7 days prior to incubation at 38°. The age of the embryo was taken as the number of days from the onset of incubation.

The mice used in this study were adult female white Albino ranging in weight from 20 to 30 g.

The pyridine, dihydropyridines, 1,4-diethoxycarbonyl-2,3,5,6-tetramethylbenzene and α-propylvaleramide were prepared previously in our laboratory.[4, 7] Hexachlorobenzene was purchased from Fisher Scientific, Fair Lawn, N. J., and coproporphyrin I tetramethyl ester (grade B) from Calbiochem, Los Angeles, Calif. Allylisopropylacetamide and methyprylon were obtained from Hoffman La Roche, Montreal, Canada; glutethimide from Dr. H. Keberle, Forschungslaboratorien, der Ciba Aktiengesellschaft, Pharmazeutische Abteilung, Basel, Switzerland; and α-isopropylvaleramide from Prof. C. Rimington, University College Hospital, London, England. Mephenytoin was supplied by Sandoz Pharmaceuticals, Dorval, Quebec, Canada; and griseofulvin by Ayerst, McKenna & Harrison, Montreal, Quebec, Canada. Sodium phenobarbital, sodium diphenylhydantoin and meprobamate were supplied by Dr. L. G. Chatten, Faculty of Pharmacy, University of Alberta; bemegride by Abbott Laboratories, Montreal, Quebec, Canada; methsuximide and phensuximide by Parke, Davis & Co., Ann Arbor, Mich. Sodium secobarbital was supplied by Eli Lilly & Co., Indianapolis, Ind.; diallylbarbituric acid by Ciba Pharmaceutical Products, Summit, N. J.; and amobarbital and aprobarbital by Drs. R. F. Labbe and M. L. Cowger, University of Washington, Seattle, Wash.

Induction of porphyria in 17-day chick embryos

The compound under study was dissolved in dimethylsulfoxide (DMSO, 0·1 ml). DMSO was selected because most of the drugs were readily soluble in this solvent and it produced no apparent toxicity. For accurate injection of this small volume, a sterile 1-in., 21-gauge disposable needle was attached to the tip of a graduated 0·2-ml pipette. A small hole was then made in the egg shell above the air sac and the drug injected through the chorioallantois into the fluids surrounding the embryo. The opening in the shell was then covered with cellophane tape and the chick embryo incubated at 38°. At the completion of the incubation period the embryo was sacrificed and the entire liver, weighing approximately 0·4 g, removed for extraction of porphyrins.

Induction of porphyria in chicks

Hexachlorobenzene was given as a 1·5% mixture by weight in the diet while griseofulvin was given as a 2·5% mixture. Feeding, to 1-day-old chicks, was *ad libitum* for 6 days. At this time the animals were sacrificed and the livers removed for analysis of porphyrins.

Induction of porphyria in mice

The compounds dissolved in saline or sesame oil (approximately 0·2 ml) were injected intraperitoneally (i.p.) once daily into mice. Insoluble compounds were suspended in sesame oil prior to injection. In one series of experiments only one injection was given. In a second series the drug was injected once daily for 4 days and in a third series once daily for 14 days. DMSO was found to be unsuitable as a solvent

for drugs as it was toxic to mice when given once daily for several days. After the desired time period animals were sacrificed and a portion (weighing approximately 0·2 g) of each lobe of the liver was removed for extraction of porphyrins.

Extraction and estimation of porphyrins in chick-embryo and mouse liver

The procedure was that described by Schwartz *et al.*[8] The liver (approximately 0·4 g) and 5 ml of ethylacetate–acetic acid (4:1) were placed in a Potter-Elvehjem apparatus and the mixture homogenized. After centrifugation of the homogenate at 2500 rpm the supernatant was decanted into a separatory funnel. The residue was resuspended in 5 ml of ethylacetate–acetic acid (4:1) and homogenized. After centrifugation the supernatant was added to the first extract in the separatory funnel. This extraction was repeated for a third time. Three % sodium acetate solution (10 ml) and 1 drop of a 0·1% solution of iodine in ethanol were added to the combined ethylacetate–acetic acid extract. After thorough agitation the lower layer containing the aqueous phase was removed and discarded. The organic layer was washed with 10 ml of 3% sodium acetate solution.

Porphyrins (coproporphyrin and/or protoporphyrin) were extracted from the organic phase with three 10-ml portions of 3 N HCl. In general where there was more than 1 μg of porphyrin per gram liver, porphyrin fluorescence was detectable by observation of the first of these extracts under a long-wave ultra-violet lamp. The volume of the combined acid extracts was made up to 100 ml with distilled water and the porphyrin content determined fluorometrically using 1 N HCl as the reagent blank.

A Turner model 110 fluorometer fitted with a 405 mμ band pass primary filter and a Wratten No. 25 (595 mμ) sharp cut secondary filter was employed for all fluorometric determinations. The latter filter passes all light above 595 mμ. A stock solution of coproporphyrin I in 1 N HCl was prepared by the procedure of Talman,[9] as modified by Schwartz *et al.*[9] An accurately weighed quantity of coproporphyrin I tetramethylester (approx. 0·5 mg) was hydrolyzed overnight with 7·5 N HCl (1 ml) and then made up to 100 ml with 1 N HCl. This stock solution was then diluted with 1 N HCl so that calibration curves covering a concentration range of 0·1 to 10 μg/100 ml were obtained. Coproporphyrin was selected because Granick[1] has shown this substance to constitute more than 80% of the total porphyrins present in induced liver cells.

Relationship between dose of DDC administered and porphyrin accumulation in liver

Chick embryo. 3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine (DDC, 0·1 mg) in DMSO (0·1 ml) was injected into five chick embryos in the manner described above. After incubation at 38° for 24 hr the liver porphyrin content was determined. This experiment was repeated using 0·5, 1, 2, 4, 8, 16 and 32 mg of DDC. A minimum of five and a maximum of ten chick embryos were used to test each dose of DDC. The results are shown in Table 1.

Mice. DDC (25 mg/kg) dissolved in sesame oil (0·2 ml) was injected i.p. into five mice. After a 24-hr period the animals were sacrificed and a portion of liver removed for porphyrin extraction. This experiment was repeated using 50, 75, 100, 125, 150, 175, 200 and 300 mg/kg of DDC. At least five mice were used to test each dose.

The rate of porphyrin accumulation in liver after injection of 4 mg DDC into chick embryos

Sixty chick embryos were injected, as described above, with 4 mg of DDC in

TABLE 1. PORPHYRIN ACCUMULATION IN LIVERS OF MICE AND CHICK EMBRYOS INDUCED BY COMPOUNDS CONTAINING PYRIDINE, DIHYDROPYRIDINE AND BENZENE RINGS*

Compound No.	Compound	17-Day chick embryo		Mice		
		Dose (mg/egg)	Porphyria-inducing activity (μg porphyrins/g liver)†	Dose (mg/kg)	Duration of experiment (days)	Porphyria-inducing activity (μg porphyrins/g liver)†
	Control (only solvent given)		0·46 (0·23–0·99)		4	0·26 (0·21–0·35)
Ia	3,5-Diethoxycarbonyl-1,4-dihydro-2,4,6-trimethylpyridine	0·1	6·2 (3·9–9·0)	25	1	6·9 (0·46–25·1)
		0·5	13·5 (3·2–16·4)	50	1	54 (1·6–175)
		1·0	19·7 (4·9–40)	75	1	34 (1·7–87)
		2·0	103 (15·0–195)	100	1	62 (3·5–135)
		4·0	136 (72–208)	125	1	70 (12·9–119)
		8·0	156 (72–327)	150	1	86 (15·4–158)
		16·0	184 (64–255)	200	1	43 (7·7–90)
		32·0	200 (65–558)	300	1	24 (4·2–86)
Ib	3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-ethyl-pyridine	0·1	6·5 (2·4–10·4)	‡		
		0·5	84 (44–204)			
		1·0	186 (102–249)			
		4·0	217 (201–247)			
Ic	3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethylpyridine	3·8	0·35 (0·32–0·38)	‡		
		7·6	0·46 (0·37–0·71)			
Id	3,5-Diethoxycarbonyl-2,4,6-trimethyl-pyridine	4·0	0·70 (0·37–0·94)	150	1	0·27 (0·24–0·29)
		8·0	1·1 (0·78–1·7)	200	1	0·22 (0·17–0·34)
		16·0	2·1 (0·36–9·7)	200	4	0·39 (0·32–0·50)
				400	4	0·24 (0·13–0·36)
				600	4	0·21 (0·14–0·26)
Ie	1,4-Diethoxycarbonyl-2,3,5,6-tetramethyl benzene	4·2	0·51 (0·32–0·72)	250	1	0·34 (0·30–0·36)
		8·4	0·42 (0·37–0·50)	500	1	0·52 (0·43–0·64)
				500	4	0·27 (0·25–0·30)

* The extreme values are given in parentheses.
† Expressed as coproporphyrin I/wet weight liver.
‡ Drug not tested.

TABLE 2. PORPHYRIN ACCUMULATION IN CHICK-EMBRYO LIVER WITH TIME AFTER INJECTION OF 4 mg DDC*

Time (hr)	Porphyria-inducing activity (μg porphyrins/g liver)†
0·5	0·37 (0·35–0·39)
3	1·10 (0·91–1·28)
6	9·1 (5·9–12·9)
10	55 (32–65)
12	43 (30–73)
16	87 (68–103)
20	112 (84–159)
24	136 (72–208)
30	173 (92–272)
36	155 (43–212)
48	179 (94–296)
Control (no drug added)	0·46 (0·23–0·99)

* The extreme values are given in parentheses.
† Expressed as coproporphyrin I/wet weight liver.

DMSO (0·1 ml). Approximately six embryos were sacrificed at each of the following time periods after administration of the drug: 0·5, 3, 6, 10, 12, 16, 20, 24, 30, 36 and 48 hr. The results are shown in Table 2.

RESULTS AND DISCUSSION

The first objective of our studies was to determine the relationship between the dose of a known porphyria-inducing drug, viz. DDC, to be used throughout as our standard of comparison, and the porphyrin accumulation in chick embryo liver. This information (Table 1) was necessary in order to make a rational selection of the range of doses of drugs which were subsequently to be tested. Our next objective was to determine the time required for a porphyria-inducing drug to induce maximal porphyrin accumulation and for this reason the rate of porphyrin accumulation after administration of DDC was determined. From the results in Table 2 it was decided to select 24 hr as an appropriate and convenient time period for studying drug-induced porphyrin accumulation since little or no significant increase occurred after this time.

The first series of compounds tested were those possessing dihydropyridine, pyridine and benzene rings. The results obtained with the dihydropyridines Ia, Ib and Ic (Table 1) in the present study, using the intact chick embryo, corresponded with the results previously obtained with the chick-embryo liver cells grown on coverslips.[1,4] Thus in both test procedures dihydropyridines Ia and Ib exhibited marked porphyria-inducing activity while Ic was inactive. On the other hand, the related pyridine compound Id and benzenoid compound Ie (Table 1) which possessed marked activity in chick-embryo liver cells grown on coverslips[7] were practically devoid of activity in the intact chick embryo (Table 1). Attempts to test 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-phenylpyridine (If) and 3,5-diethoxycarbonyl-1,4-dihydro-4-methylpyridine (Ig) in the chick embryo were unsuccessful because of their toxicity.

The results obtained with secobarbital, diallylbarbituric acid, phenobarbital, allylisopropylacetamide (AIA) and α-propylvaleramide (Tables 3 and 4), using the intact chick embryo, corresponded to the results previously obtained with the chick-

TABLE 3. PORPHYRIN ACCUMULATION IN LIVERS OF MICE AND CHICK EMBRYOS INDUCED BY BARBITURATES AND RELATED COMPOUNDS*

Compound	17-Day chick embryo		Mice		
	Dose (mg/egg)	Porphyria-inducing activity (μg porphyrins/g liver)†	Dose (mg/kg)	Duration of experiment (days)	Porphyria-inducing activity (μg porphyrins/g liver)†
Control (only solvent given)		0·46 (0·23–0·99)		4	0·26 (0·21–0·35)
Allylisopropyl-acetamide	1·0	9·7 (0·77–28)	‡		
	4·0	33 (10·7–106)			
	8·0	25 (7·4–46)			
α-Propylvaleramide	2·2	10·6 (0·68–30)	250	1	0·30 (0·28–0·34)
	4·3	33 (0·74–152)	250	4	0·28 (0·18–0·42)
	8·6	5·8 (0·80–25)	500	1	0·45 (0·24–0·60)
			500	4	0·34 (0·31–0·37)
α-Isopropylval eramide	4·0	4·3 (1·6–15·5)	‡		
	8·0	0·80 (0·75–1·3)			
Sodium secobarbital	3·9	10·1 (0·65–45)	50§	1	0·21 (0·20–0·24)
	7·8	20·3 (11·3–37)	50	4	0·25 (0·22–0·28)
			100	1	0·31 (0·22–0·42)

* The extreme values are given in parentheses.
† Expressed as coproporphyrin I/wet weight liver.
‡ Drug not tested.
§ Secobarbital was administered in saline; control values are 0·19 (0·17–0·21).

TABLE 4. PORPHYRIN ACCUMULATION IN LIVERS OF CHICK EMBRYOS INDUCED BY BARBITURATES*

Compound	17-Day chick embryo	
	Dose (mg/egg)	Porphyria-inducing activity (μg porphyrins/g liver)†
Sodium amobarbital	3·7	0·67 (0·55–0·79)
	7·4	0·66 (0·49–1·0)
	14·8	3·1 (1·1–4·8)
Aprobarbital	3·5	4·1 (0·73–14·1)
	7·0	31 (1·1–81)
Diallylbarbituric acid	3·1	0·63 (0·55–0·69)
	6·2	0·80 (0·39–1·3)
	12·4	6·4‡
Sodium phenobarbital	3·8	0·69 (0·57–1·0)
	7·6	1·1 (0·57–2·5)
	15·2	1·2 (0·86–1·6)

* The extreme values are given in parentheses.
† Expressed as coproporphyrin I/wet weight liver.
‡ Only one animal out of five survived this dose.

embryo liver cells.[5, 7, 10] Thus in both test procedures secobarbital, AIA and α-propylvaleramide exhibited marked porphyria-inducing activity while phenobarbital[1] and diallylbarbituric acid had weak activity.[5] On the other hand, the related compound α-isopropylvaleramide which had comparable activity to AIA in chick-embryo liver cells was considerably less active than AIA in the intact chick embryo. Aprobarbital which possessed marked activity and amobarbital which was weakly active have not been tested in the chick-embryo liver cells. In this group of compounds previous workers[6, 11, 12] have emphasized the importance of an allyl group in a drug for the demonstration of porphyria-inducing activity in intact animals and chick embryos. Studies of Hirsch *et al.*[5] showed that an allyl group was not required for activity in this group of compounds when using chick embryo liver cells grown on coverslips. Our present results with α-propylvaleramide and α-isopropylvaleramide in the intact chick

TABLE 5. PORPHYRIN ACCUMULATION IN LIVERS OF MICE AND CHICK EMBRYOS INDUCED BY MISCELLANEOUS DRUGS*

Compound	17-Day chick embryo		Mice		
	Dose (mg/egg)	Porphyria-inducing activity (μg porphyrins/g liver)†	Dose (mg/kg)	Duration of experiment (days)	Porphyria-inducing activity (μg porphyrins/g liver)†
Control (only solvent given)		0·46 (0·23–0·99)		4	0·26 (0·21–0·35)
Glutethimide	1·0	0·47 (0·27–0·64)	160	4	0·27 (0·23–0·32)
	4·0	4·9 (0·84–13·1)	160	14	0·30 (0·26–0·36)
	6·6	37 (3·5–91)	200	1	0·49 (0·42–0·56)
Mephenytoin	4·0	7·4 (0·79–27)	160	4	0·28 (0·21–0·38)
	8·0	1·9 (1·3–3·4)	160	14	0·36 (0·28–0·46)
			200	1	0·28 (0·23–0·39)
Methyprylon	4·0	4·5 (1·1–12·8)	135	4	0·36 (0·27–0·44)
	8·4	26 (0·94–121)	135	14	0·30 (0·20–0·51)
			400	4	0·27 (0·22–0·33)
			600	1	0·56 (0·31–0·86)
Sodium diphenyl-hydantoin	4·1	0·42 (0·34–0·60)	25	4	0·29 (0·22–0·39)
	8·2	1·2 (1·1–1·3)	50	4	0·57 (0·43–0·77)
			100	1	0·39 (0·35–0·51)
			100	3	0·34 (0·26–0·40)
			150	1	0·30 (0·20–0·36)
Meprobamate	3·3	1·6 (0·72–5·8)	250	1	0·22 (0·17–0·24)
	6·6	3·3 (1·2–6·7)	500	1	0·26 (0·18–0·33)
			500	4	0·23 (0·18–0·26)
Bemegride	4·6	0·63 (0·50–0·76)	25	1	0·40 (0·36–0·51)
			25	14	0·25 (0·20–0·30)
Methsuximide	4·0	18·2 (2·2–47)	150	4	0·27 (0·22–0·32)
	8·0	37 (0·75–72)	150	14	0·34 (0·31–0·43)
Phensuximide	4·0	0·82 (0·45–1·2)	140	4	0·45 (0·41–0·51)
	11·4	4·5 (0·99–7·6)	150	14	0·24 (0·16–0·32)
			600	1	0·25 (0·20–0·31)
Griseofulvin	4·0	0·53 (0·41–0·64)	‡		
	8·0	0·68 (0·47–0·92)			
	16·0	0·52 (0·09–1·2)			
Hexachlorobenzene	5	0·43 (0·39–0·46)	‡		

* The extreme values are given in parentheses.
† Expressed as coproporphyrin I/wet weight liver.
‡ Drug not tested.

embryo show that an allyl group is not essential for activity, but does enhance the activity when present.

Using the intact chick embryo, porphyria-inducing activity was revealed in the following compounds in agreement with results obtained in the chick-embryo liver cells (Table 5): glutethimide, mephenytoin, methyprylon, meprobamate, methsuximide, phensuximide and diphenylhydantoin. However, bemegride, griseofulvin and hexachlorobenzene which were shown to be active in isolated liver cells[1] could not be shown to be active in the intact embryo. In the case of bemegride this discrepancy might be explained by the fact that it was toxic in the chick embryo and a large enough dose could not be used to demonstrate activity. Hexachlorobenzene was only slightly soluble in DMSO and this might have accounted for the failure to demonstrate activity with this compound. In summarizing our results it is apparent that comparable results in the two test systems were obtained with fifteen out of twenty-one compounds tested. Thus in the case of the majority of drugs tested the chick-embryo liver cells grown as a monolayer culture responded in the same way as the liver of the intact chick embryo.

Hexachlorobenzene and griseofulvin have previously been shown to induce porphyria in man[13] and in several animal species.[14, 15] It was thus of interest that these compounds were active in the chick-embryo liver cell system but inactive in the intact embryo. There appeared to be two possible explanations for this finding: (1) the isolated liver cells grown in monolayer culture possessed altered properties when compared to the intact liver cells, or (2) the drugs were unable to reach the liver after injection into the chick embryo. It was thus desirable to test whether porphyrin accumulation, in the liver, could be induced when these drugs were given orally to young chicks for 6 days. By this means porphyria-inducing activity was detected in both drugs (Table 6). It is likely therefore that these drugs after injection into the fluids surrounding the chick embryo did not reach the liver in sufficient quantity to induce porphyrin accumulation.

TABLE 6. PORPHYRIN ACCUMULATION IN THE LIVERS OF YOUNG CHICKS AFTER ORAL ADMINISTRATION OF DRUGS*

Compound	% Drug administered in diet	Porphyria-inducing activity (μg porphyrins/g liver)†
Hexachlorobenzene	1·5	0·83 (0·70–0·93)
Griseofulvin	2·5	0·34 (0·32–0·38)
Control	0	0·16 (0·11–0·19)

* The extreme values are given in parentheses.
† Expressed as coproporphyrin I/wet weight liver.

Our next objective was to observe the porphyria-inducing activity of some of these drugs in mice. This was desirable in order to see how the response of avian liver cells compared to those of mammalian liver cells. The relationship between the dose of DDC to be used as our standard of comparison and the porphyrin accumulation in mouse liver was determined (Table 1). While DDC (Ia) was demonstrated by this means to cause accumulation of large quantities of porphyrin (coproporphyrin and/or

protoporphyrin) the other drugs tested were shown to have negligible activity in this regard (Tables 1, 3 and 5). We have assumed that porphyrin accumulation in the liver can be equated with increased synthesis of δ-aminolevulinic acid synthetase in view of the fact that Granick[1] has marshalled considerable evidence to support this idea in chick-embryo liver cells. While it is possible that this assumption does not hold in the intact animal where there is the possibility of excretion of porphyrins and precursors, there have been no reports of porphyria-inducing drugs which failed to cause the accumulation of coproporphyrin and/or protoporphyrin in the liver.[16, 17] In view of the failure to demonstrate porphyria-inducing activity in mice the question is raised as to the relevance of results obtained with an avian species to the results anticipated in the porphyric patient. To resolve this question it is valuable to consider results obtained with some of the above drugs when administered to patients with porphyria. Thus secobarbital, meprobamate, methsuximide and diphenylhydantoin which have been shown to produce a clinical relapse in porphyric patients[18] are active in the avian liver using isolated cells or the intact chick embryo. Since these drugs appear to be inactive in mice it seems that the results obtained using the avian liver cells allow better prediction of the results to be expected in the porphyric patient than the results in mice. The reasons for this are not clear and require further investigation.

The test system designed by Granick[1] utilizing chick-embryo liver cells has several important advantages. The liver is examined independently of all other body tissues and permeability problems are reduced. In our laboratory we have found this technique invaluable in the study of the mechanism of action of porphyria-inducing drugs. However, in laboratories which are not equipped for cell culture studies considerable effort is required to test drugs by this procedure. By contrast the system utilizing the intact chick embryo requires equipment available to most routine clinical laboratories, and since it may be carried out with the expenditure of very little time and money, would appear to be a useful practical procedure for screening drugs prior to administration to porphyric patients. It must however be borne in mind that this procedure will not reveal porphyria-inducing activity in all compounds tested.

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REFERENCES

1. S. Granick, *J. biol. Chem.* **241**, 1359 (1966).
2. S. Granick, *J. Am. med. Ass.* **190**, 475 (1964).
3. C. J. Watson, *J. Am. med. Ass.* **197**, 1074 (1966).
4. G. S. Marks, E. G. Hunter, U. K. Terner and D. Schneck, *Biochem. Pharmac.* **14**, 1077 (1965).
5. G. H. Hirsch, J. D. Gillis and G. S. Marks, *Biochem. Pharmac.* **15**, 1006 (1966).
6. E. L. Talman, R. F. Labbe and R. A. Aldrich, *Archs Biochem. Biophys.* **66**, 289 (1957).
7. G. H. Hirsch, G. L. Bubbar and G. S. Marks, *Biochem. Pharmac.* **16**, 1455 (1967).
8. S. Schwartz, M. H. Berg, I. Bossenmaier and H. Dinsmore, in *Methods of Biochemical Analysis* (Ed. D. Glick) vol. 8, p. 221. Interscience Publishers, New York (1960).
9. E. L. Talman, in *Standard Methods of Clinical Chemistry* (Ed. D. Seligson) vol. 2, p. 137. Academic Press, New York (1958).
10. D. W. Schneck, W. J. Racz, G. H. Hirsch, G. L. Bubbar and G. S. Marks, *Biochem. Pharmac.* **17**, 1385 (1968).
11. W. Stich and P. Decker, in *Ciba Foundation Symposium on Porphyrin Biosynthesis and Metabolism* (Eds. G. E. W. Wolstenholme and E. C. P. Millar) p. 254. Churchill, London (1955).

12. A. GOLDBERG and C. RIMINGTON, in *Diseases of Porphyrin Metabolism*, p. 192. Thomas, Springfield (1962).
13. F. DE MATTEIS, *Pharmac. Rev.* **19**, 523 (1967).
14. P. K. OCKNER and R. SCHMID, *Nature, Lond.* **189**, 499 (1961).
15. C. RIMINGTON, *Br. J. Dermatol.* **75**, 91 (1963).
16. J. ONISAWA and R. F. LABBE, *J. biol. Chem.* **238**, 724 (1963).
17. F. DE MATTEIS and B. E. PRIOR, *Biochem. J.* **83**, 1 (1962).
18. M. L. COWGER and R. F. LABBE, *Lancet* **i**, 88 (1965).