Effect of Sedormid on the Developing Chick Embryo.* (20955)

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The ability of Sedormid (allylisopropylacetyl carbamide) to produce acute porphyria in man was suggested by the findings of Duesberg(1). A similar disorder has recently been induced in rabbits(2) and rats(3) by the oral administration of the same drug. The purpose of the present communication is to report the appearance of abnormal amounts of porphyrin-like substances in embryonated eggs receiving Sedormid in the yolk sac or in the allantoic cavity.

Materials and methods. White Leghorn eggs were used in all experiments. Sterile suspensions of Sedormid were prepared in final concentrations of 20 to 40 mg/ml as follows: 1) Weighed samples of Sedormid were suspended in isotonic (0.3 molar) glycerol containing 0.1% Tween 80 in a mortar or a Waring blendor. Suitable volumes of the suspension were transferred to vaccine vials and sterilized by heating in a boiling water bath for one hour on each of 3 successive days. The vials were shaken frequently during heating and upon removal were placed in a shaking machine until cool. 2) Weighed samples of Sedormid were transferred to cotton stoppered flasks and sterilized in a hot air oven at 160°C for one hour. After sterilization of the glycerol-Tween 80 solution by Seitz filtration, the 2 reagents were homogenized in a sterile mortar and transferred to sterile vaccine vials. Appropriate volumes of such sterile suspensions were injected into the yolk or allantoic sacs of 8-day embryonated eggs in the manner usually employed for viral inoculations. The maximum volume given was 0.75 ml. Control eggs received 0.75 ml of glycerol-Tween 80 at the same time. Eggs were incubated at 37°C and candled daily for viability. At convenient intervals after inoculation several eggs from each group were chilled at 0°C for one hour to prevent bleeding, the shell over the air sac cut away and the allantoic fluids aspirated with a syringe and needle. Each fluid was harvested separately, centrifuged and a measured aliquot was included as part of a final pool for any given lot of eggs. Pools were composed of allantoic fluids from 3 to 6 living embryos. After addition of 3 ml of 10% trichloroacetic acid to 5 ml of each pool and removal of the precipitate by centrifugation, the supernate was adjusted to pH 6-7 with NaOH and brought to a volume of 10 ml. A 5-ml aliquot of this solution was used for the determination of coproporphyrin by the method of Schwartz *et al.*(4). Uroporphyrin was estimated in the aqueous phase from the coproporphyrin determination as outlined by Case *et al.*(3). Since allantoic fluid contains little protein if trauma to blood vessels is avoided, an estimate of the total amount of fluorescent material present was obtained by treating 2.5 ml of the pool with 5 ml of 7.5 N HCl, removing the precipitate by centrifugation, diluting the supernatant to 25 ml with distilled water, and reading the resulting solution in the fluorimeter against a coproporphyrin standard.

Results. The data which were obtained at varying intervals following the inoculation of 15 mg of Sedormid per embryo via the allantoic cavity and via the yolk sac are presented in Table I. The results in the first 2 columns are expressed in terms of a coproporphyrin standard either as μg of uro or coproporphyrin per 100 ml of pooled allantoic fluid. The third column indicates the sum of these 2 determinations and is compared with the values for total porphyrin as measured by the technic given. Elevated porphyrin levels were first noted 64 hours after the introduction of the drug into the embryos and these were maintained for the duration of the experiment

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TABLE I. Porphyrin Levels in Allantoic Fluids of Chick Embryos Given 15 mg Sedormid: Yolk Versus Allantoic Cavity as Route of Inoculation.

Incubation, hr	Control				Inoculation route yolk sac				Allantoic cavity			
	Uro*	Copro	Sum	Total	Uro*	Copro	Sum	Total	Uro*	Copro	Sum	Total
40	0	1	1	—	0	3	3	10	0	1	1	—
64	0	1	1	3	7	15	22	23	13	27	40	53
88	0	3	3	4	29	77	106	—	25	68	93	—
112	0	2	2	2	26	54	80	96	36	93	129	129
160	1	5	6	10	50	61	111	138	72	136	208	183
184	9	2	11	14	420	270	690	520	220	180	400	454
234	14	2	16	21	295	95	390	308	375	353	728	630

Values expressed as μg of coproporphyrin/100 ml allantoic fluid.
* Unheated specimens.

170 hours later. The values for total fluorescence showed a similar rise.

In the second experiment varying doses of Sedormid were given into the allantoic cavity of 8-day embryos and the allantoic fluids were harvested at 3 intervals as shown in Table II. Assays for total porphyrin were obtained at 40, 112, and 160 hours; partition into urotype and coproporphyrin was done on the 112-hour pool only. A distinct elevation in total fluorescence was noted at the end of 40 hours and with as little as 5 mg Sedormid per egg. The 10-mg dose was as effective in eliciting a maximal response as larger amounts of drug and was less toxic to the embryos. With time there was a gradual and progressive accumulation of porphyrin-like substances in the allantoic cavity. A portion, but not all, of the increase may be accounted for by the reduction in the volume of allantoic fluid which normally occurs between the 14th and 16th day of embryonic development.

The outstanding effects of Sedormid on the developing chick were a decrease in size of the embryo and the appearance of edema. Three groups of embryos are shown in Fig. 1,

TABLE II. Porphyrin Levels in Allantoic Fluids of Embryos Given Varying Doses of Sedormid.

Dosage (mg)	40 hr pool total	112 hr pool				160 hr pool total
		Uro	Copro	Sum	Total	
30	23	64	80	144	147	—
20	43	100	80	180	168	435
15	32	60	66	126	108	263
10	40	100	97	197	138	428
5	15	18	26	44	27	345
1	5	0	1	1	3	30
0	3	—	—	—	—	10

μg/100 ml allantoic fluid.

160 hours after the inoculation of 15 ml of Sedormid. The greatest stunting was observed in the eggs injected into the yolk sac (Y) and the 3 illustrated weighed a total of 19 g. Those receiving the drug into the allantoic cavity (A) were slightly larger and weighed 22 g. The control embryos (C) totaled 30 g. In addition doses of 20 and 30 mg of Sedormid induced a marked edema which was generalized in character. At this level of drug significant degrees of hemorrhage were noted over the surface of the embryos and occasionally in the brain itself. The organs were not grossly altered but the livers were friable and some were dark green in color. Those embryos which manifested edema were extremely friable indicating that the changes could have been terminal even though the chicks were alive at the time the allantoic fluids were harvested. Examination of the chicks under the ultraviolet light revealed gross, red fluorescence in the kidneys, sternum, and long bones. Studies of tissue components will be reported separately.

Discussion. Protoporphyrin has been identified in the eggshell(5,6), and eggshell membranes(7), in the yolk(8), and in the albumin(9) of the developing hen's egg. Schønheyder(10) has crystallized coproporphyrin I isolated from chick embryos. No studies dealing with porphyrins in allantoic fluid have been reported.

The general pattern of the response of chick embryos to Sedormid treatment corresponds to that of rabbits(2) and rats(3). However, since none of the porphyrins have been isolated for spectrophotometric study and identification of their isomeric configurations, the

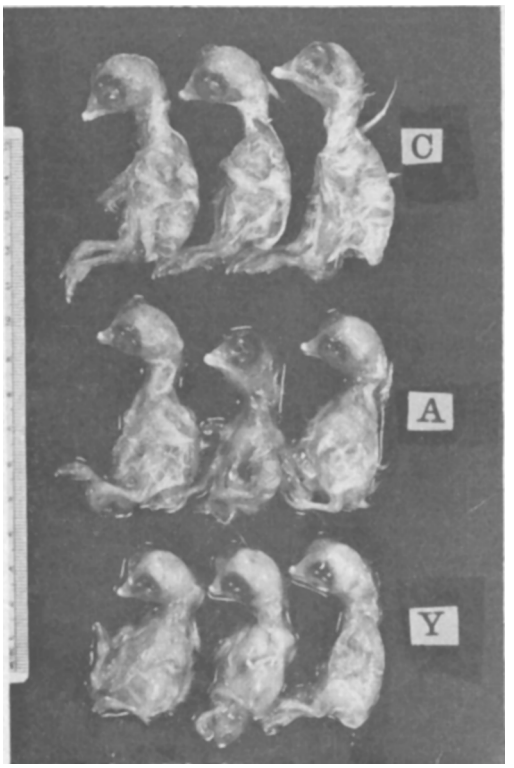

FIG. 1. Chick embryos given Sedormid. C = controls; A = 15 mg intra-allantoic; Y = 15 mg via yolk sac.

production of an experimental porphyria in chick embryos has not yet been established. The data thus far obtained establish only that Sedormid produces a metabolic disturbance resulting in the appearance of considerable quantities of fluorescent material in the allantoic fluids of treated eggs within a very short time. The fluorescent materials observed in both control and Sedormid-treated eggs will probably prove to be porphyrins but further interpretations based upon these data are not justified until the porphyrins present have been isolated and identified and their isomer types established. If the porphyrins from Sedormid-treated eggs are of the proper isomer type (Type III) to allow this disorder to be labeled acute porphyria, the chick embryo offers several advantages for studies of porphyrin metabolism, one of the most important of these being its status as a convenient, inexpensive, bacteria-free system.

Summary. The inoculation of Sedormid into the yolk sacs or allantoic cavities of 8-day-old embryonated eggs results in the prompt appearance in their allantoic fluids of materials exhibiting red fluorescence in ultraviolet light. These fluorescent materials appear to accumulate progressively with time. The results of quantitative estimations suggest that coproporphyrin and uroporphyrin are responsible for this fluorescence. Treated embryos are smaller than the controls and exhibit red fluorescence of the kidneys, sternum, and long bones under ultraviolet light. Further interpretation of these data must await isolation and characterization of the fluorescent substances.

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