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Some Antagonistic Effects of "Hematoporphyrin" and Protoporphyrin in Mice.* (29259)

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The companion report(1) has described certain physiological changes which followed administration of lethal or near-lethal doses of "hematoporphyrin" ("Hp") to mice and rabbits. Hypotension with associated decrease in peripheral blood flow and depression of total body oxygen uptake were especially prominent phenomena in these animals. The attempts to modify or prevent these effects, as described in the present report, were undertaken with 3 ultimate objectives in mind: a) to gain information regarding possible mechanisms involved, b) to investigate possible antidotes which might counteract "hematoporphyrin" toxicity in man or animals receiving large doses of porphyrin prior to x-irradiation of their tumors(2,3), and c) to reduce adverse side effects or enhance desirable effects related to tumor regression in subjects treated with a combination of porphyrin and x-irradiation.

Seventeen compounds were chosen for testing because of reports on their modification of cardiovascular response or oxygen uptake. Protoporphyrin was tested because of reports by Granick and Gilder(4,5) that "Hp" acts as a metabolite antagonist to protoporphyrin in *Hemophilus influenzae* which require the latter porphyrin for growth. This antagonism is based on the close similarity of the 2 molecules, "Hp" having 2 hydroxyethyl groups in place of the 2 vinyl groups of protoporphyrin. It seemed possible that the reverse, namely antagonism of "Hp" by protoporphyrin, might also be demonstrable.

Materials and methods. Unless otherwise noted, the "hematoporphyrin" ("Hp") employed was obtained from Mann Research Laboratories. The "Hp" obtained from Merck Sharp and Dohme (MSD "Hp") was a different preparation from that used in our earlier mortality studies(1). The protoporphyrin was prepared from hemoglobin by the method of Grinstein(6). Its methyl ester was purified by chromatography on calcium carbonate and recrystallized several times. Following hydrolysis in 7 N HCl, it was precipitated by addition of excess sodium acetate (saturated aqueous solution), washed with water and dried. Other materials tested were obtained from commercial sources.

The porphyrins were dissolved in 2% sodium bicarbonate for parenteral administration as noted below. Other compounds tested (Table I) were generally administered subcutaneously in saline solution. They were given either as single doses immediately after injection of the "Hp" or in divided doses 3 times daily.

ZBC strain of female mice weighing 17-22 g were used. Unless noted otherwise they were kept in a room illuminated only by a 25 watt red light to avoid photodynamic effects. They were examined 3 times daily for 7 days, then daily for an additional 21 days for survival. Total body oxygen uptake was measured with the previously described respirometer(1).

Results. Neither single nor multiple injections of the 17 compounds listed in Table I had a significant effect on the survival of mice administered an LD_{90} to MLD_{100} of "Hp"

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TABLE I. Compounds Which Failed to Modify Survival of Mice Administered an LD_{90} to MLD_{100} of "Hematoporphyrin."*

Compound	Dose (mg/mouse) †
1. Metaraminol	.001, .0005, .0002
2. Atropine sulfate	.002, .0005, .0002
3. Calcium disodium versenate	9.0, 3.0, 1.0
4. Chlorpromazine HCl	.01, .005, .0025
5. Chlorpheniramine maleate	.02, .008, .005, .003, .001
6. Hydrocortisone	1.0, .25, .05
7. 2,4-Dinitrophenol	.1, .5
8. 2,2-Dinitropropane	.1, .5
9. 2,4-Dinitro-6-phenylphenol	.1, .5
10. 2,5-Dinitrofluorene	.1, .5
11. 2,4-Dinitro-6-sec. butylphenol	.1, .5
12. Heparin sodium	.30, .15, .075
13. Methylene blue	2, .1, .03, .01
14. Papaverine HCl	1.0, .2, .04, .01
15. Neostigmine	.01, .005, .002, .001
16. Serotonin	.01, .005, .002, .0001
17. Promazine HCl	.02, .005

All compounds administered subcutaneously. Compounds 7-11 were administered in peanut oil; the remainder were dissolved in saline.

* .5 Mg "Hp" (MSD) per gram body weight; single dose.

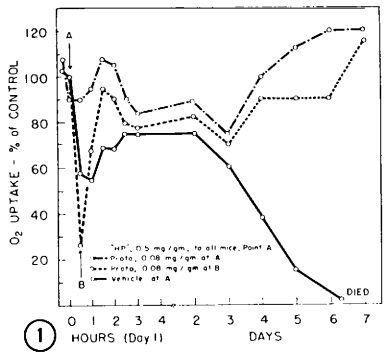
† 1-3 Doses per day.

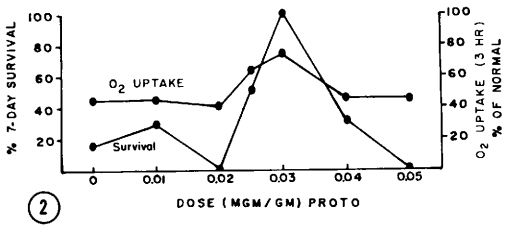
(MSD). A total of 709 mice (at least 10 per group) was employed for these studies.

Oxygen uptake and survival were studied in 18 mice injected intravenously with 0.025 to 0.25 mg of protoporphyrin per gram body weight. The observed MLD_{100} was 0.22 mg per gram. No consistent variation from normal was seen in the pattern of oxygen uptake regardless of the dose of protoporphyrin. In half the animals values remained within 25% of pre-injection levels, in 5 they fell to about 50% of control levels for only 2 or 3 hours, and in 4 they reached minimum values after 24 hours. In 3 mice, values rose to 150% or more of control levels 3 to 4 days after administration of the protoporphyrin.

Protoporphyrin injected subcutaneously proved to be much less toxic. Six mice which received 0.55 mg per gram body weight by this route all survived, and only one of 6 died following injection of 0.60 mg per gram. Oxygen uptake was not measured in these animals.

The effect of protoporphyrin on oxygen uptake and survival of mice administered an approximate LD_{90} of "Hp" was next studied. In a preliminary experiment, 6 mice were injected intraperitoneally with 0.5 mg per gram of "Hp". Immediately thereafter 2 of these mice were injected intravenously with 0.08 mg of protoporphyrin per gram body weight (approximately ⅓ its MLD_{100}), while 2 mice received the same amount of protoporphyrin 30 minutes after the "Hp" injection. The remaining 2 mice received 0.5 ml of two per cent sodium bicarbonate. As shown in the average data plotted in Fig. 1, the protoporphyrin exerted a protective effect as demonstrated by the maintained or increased





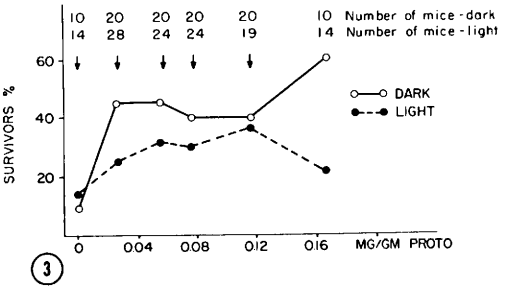


FIG. 1. Protective effect of protoporphyrin (I.V.) in mice administered "hematoporphyrin" (I.P.).

FIG. 2. Survival and oxygen uptake of mice administered various amounts of protoporphyrin (I.V.) plus 0.4 mg "Hp"/gram body weight (I.P.).

FIG. 3. Protective effect of protoporphyrin in "Hp"-administered mice; comparative results in lighted and darkened rooms. All mice were administered "Hp," 0.5 mg/gram body weight plus varying amounts of protoporphyrin or of vehicle alone. Both porphyrins were injected subcutaneously.

oxygen uptake and prolonged survival.

Another 30 mice received 0.4 mg of "Hp" per gram body weight intraperitoneally, followed immediately by an intravenous injection of either vehicle alone or of 0.01 to 0.05 mg of protoporphyrin per gram body weight. Oxygen uptake was measured prior to and for 3 hours following the porphyrin injections. The animals were followed 7 days for survival. As shown in Fig. 2, optimum protection followed administration of 0.03 mg of protoporphyrin per gram body weight.

The possible influence of moderate light exposure on the protective effect of protoporphyrin was investigated in 223 mice. One hundred mice were kept in rooms illuminated only by a 25 watt red light while the remaining mice were kept in a room illuminated constantly and only with fluorescent ("cool white") bulbs. All the mice were administered 0.5 mg of "Hp" per gram body weight and varying amounts of protoporphyrin or of vehicle. All injections were given subcutaneously. As seen in Fig. 3, protection by protoporphyrin was evident in both groups, but was more marked in mice kept in darkness. Three of 24 control mice (12%) survived after receiving "Hp" and vehicle only. (Of 54 mice given the same dose of "Hp" in other experiments not described here 15% survived, giving an overall survival of 14% in 78 mice administered this dose of "Hp" subcutaneously.) Of the 199 mice which received any additional amount of protoporphyrin, 47% survived when kept in darkness while 30% survived in moderate light. The overall survival in these two latter groups was 36%.

Discussion. The unique modification by protoporphyrin of "Hp" toxicity in mice is not easily explained, especially since the precise factors involved in the production of hypotension and decreased O_2 uptake by "Hp" are unknown.

Granick and Gilder's conclusions(4) regarding the metabolite antagonism behavior of "Hp" and protoporphyrin in *Hemophilus influenzae* are of special interest in relation to the interpretation of this study. The finding that protoporphyrin action was antagonistic to that of "Hp" in the mice while "Hp" action was antagonistic to that of protoporphyrin in their system, as well as the apparent dose-dependent nature of the results in mice given protoporphyrin intravenously (Fig. 2) suggest a metabolite antagonism mechanism. It is of interest, too, that Granick and Gilder reported an inhibiting ratio of 1:10 (proto: "HP") in their study while approximately the same optimum inhibiting ratio was found in these mice. In their system, "Hp" apparently acted to inhibit the synthesis of required protoheme, while the rapidity of the protoporphyrin effect in "Hp"-treated mice makes this type of inhibition unlikely. It suggests rather, a direct chemical effect by protoporphyrin on the primary reactions elicited by "Hp" administration.

Both respiratory and glycolytic enzymes are inhibited variously by different porphyrins(7,8). Unpublished studies‡ have shown that "Hp" and protoporphyrin may exert opposite effects on the oxidation of $NADPH_2$ in an Ehrlich ascites tumor system. Studies employing variable mixtures of these 2 porphyrins in this or other enzyme systems have not, to our knowledge, been performed.

In the studies summarized in Fig. 1 and 2 the protoporphyrin was injected intravenously and the mice were kept in a 100% oxygen atmosphere during the first 3 hours. In the study shown in Fig. 3 the protoporphyrin was injected subcutaneously and the mice were kept in air. Further studies are planned to determine whether different oxygen tensions shortly after the porphyrin injections or the route of administration are of major significance in accounting for the different patterns of response observed with various doses of protoporphyrin.

The possible role of tissue anoxia in limiting the response of tumors to combined treatment with "hematoporphyrin" or its derivatives and x-irradiation is of special importance, since those porphyrins which enhance radiosensitivity do so only when present in very low concentration. At high concentrations the enhancing effect is lost and the porphyrin may become radioprotective(3). Whether protoporphyrin can counteract this effect or

‡ Fabiny, Schwartz, Wattenberg, to be published.

whether other factors are involved remains to be determined.

Summary. Of 18 compounds studied, only protoporphyrin significantly protected mice injected with lethal doses of "hematoporphyrin." Results of separate experiments with 277 mice showed (a) 14% survival in 76 mice which received "Hp" and vehicle alone, (b) 47% survival in 90 mice injected with protoporphyrin in addition to the "Hp" and kept in the dark, and (c) 30% survival in 109 mice injected with both porphyrins and kept in a room illuminated with fluorescent lights. The marked fall in total body oxygen uptake associated with "Hp" administration was decreased considerably by administration of protoporphyrin.

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On the Mechanism of Emesis Induced by 5-Hydroxytryptamine. (29260)

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The author(1) has reported that intravenous injection of catecholamines induces emesis in cats and dogs. Recently, Peng(2), has confirmed these results. Studies of a similar nature with 5-hydroxytryptamine (5HT) have yielded seemingly contradictory results. Bogdanski *et al*(3) demonstrated that injection of 5-hydroxytryptophan (5HTP), the precursor of 5 HT, produces vomiting in dogs; on the other hand, Feldberg and Sherwood(4) reported that 5HT introduced into the ventricles of cats does not elicit this effect.

The experiments described here were carried out to determine whether 5HTP-induced emesis in cats might be mediated through the release of brain norepinephrine (NE).

Materials and methods. Experiments were carried out in nonfasted cats of either sex, weighing 1.5-4.0 kg. The animals received 50 ml of lukewarm water by stomach tube and 20 minutes later were given various doses of 5HTP (1% solution in isotonic saline). The emetic effects of 5HTP were determined in control cats and in cats pretreated with various monoamine oxidase (MAO) inhibitors (iproniazid, nialamide and isocarboxazid), with central depressants (pentobarbital and chlorpromazine) and with various NE-depleting agents (α-methyl-3-4 dihydroxyphenylalanine [α-methyl DOPA], α-methyl-m-tyrosine [α-MMT] and reserpine)(5,6). The results are expressed as the proportion of cats that showed an emetic effect. All drugs were administered intraperitoneally unless otherwise noted.

Results. Emesis produced by 5HTP. Table I shows that the emetic effect of 5HTP (10 to 50 mg/kg, i.p.) was related to the dosage; in doses of 50 mg/kg, 5HTP produced vomiting in 5 of 5 cats. No vomiting occurred in 8 cats given saline alone. Tolerance to 5HTP did not occur, for an almost identical dose-response curve was obtained when the amino acid was given to the animals 5 days later. In contrast to the catecholamines which in-