Oxygen Uptake and Cardiovascular Response of Mice and Rabbits Administered "Hematoporphyrin."* (29258)

J. MODELL† AND S. SCHWARTZ
Department of Medicine, Medical School, University of Minnesota, Minneapolis

Studies on the modification of radiosensitivity of tumors by porphyrins in man(1) and animals(2)‡ made it necessary to investigate certain physiological and biochemical effects of the porphyrins employed. "Hematoporphyrin" ("Hp") from 6 different suppliers was found to be a mixture of 7 to 12 or more different porphyrins, separable by Craig countercurrent or column chromatography techniques.‡ In these original crude preparations, hematoporphyrin comprised 38 to 64% of the total porphyrin. These "hematoporphyrins" and some of the subfractions isolated from them, were administered parenterally to mice and rabbits to determine their lethal doses and to observe their effect on oxygen uptake and some related physiological processes. Oxygen uptake of liver homogenates to which "hematoporphyrin" was added was also measured. The changes observed will be described in this report, and attempts to counteract them will be considered in the companion paper(3).

Materials and methods. Unless specified otherwise, the studies to be described employed "Hp" obtained from Mann Research Laboratories, N. Y., as the repeatedly recrystallized hydrochloride. By countercurrent analysis, 64% of the total appeared to be hematoporphyrin. The porphyrins were generally dissolved directly in 2% sodium bicarbonate shortly before use. In some instances they were dissolved in 1% sodium hydroxide and the solution was then brought to pH 7.6-7.8 by titration with 1% hydrochloric acid. They were administered by various routes as noted below. To minimize effects related to changing toxicity with variation in circadian system phase, all injections were made from 8 a.m. to 12 o'clock noon.

Female ZBC mice, generally weighing 17-22 g, were used in all the studies to be described. Because of the marked photodynamic effect produced by these porphyrins, the mice were kept in rooms illuminated only by a 25 watt red light. During the 30 days following porphyrin administration they were examined at least once daily for survival or for additional studies.

Total oxygen uptake was measured in individual mice placed in the respirometer shown in Fig. 1. The respirometer was primed and maintained at atmospheric pressure with oxygen. Soda lime cannisters for carbon dioxide absorption were changed prior to each study. After repeated measurements of oxygen uptake, the porphyrin solution (or an equal volume of vehicle alone) was injected and measurements continued every 15 minutes for 3 hours or more. Thus each animal served as its own control. The mice generally remained quiet within the respirometer. Experiments were terminated and results discarded in the few instances where excessive fluctuation of control oxygen uptake values was observed.

Total oxygen uptake, systolic blood pressure, respiratory rate, and blood oxygen content were determined in 4 rabbits prior to and following intravenous administration of "hematoporphyrin." Each rabbit was connected *via* a tracheostomy tube to an oxygen reservoir similar in principle to that shown in Fig. 1. One soda lime and 2 sodium hydroxide traps were used to absorb expired carbon dioxide. Blood pressure was measured directly with a mercury manometer attached to a cannula in the left femoral artery. Arterial and venous blood samples were obtained through polyethylene catheters inserted into the right common iliac artery and right jugular vein (or inferior vena cava) re-

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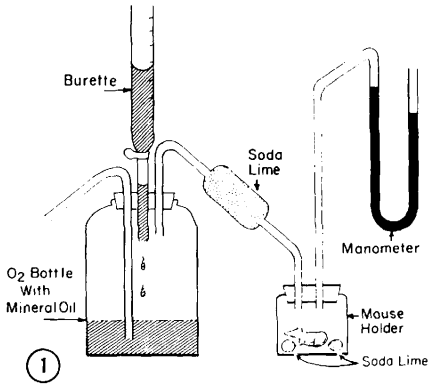
† Present address: Dept. of Anesthesiology, Univ. of Miami, School of Med., Jackson Memorial Hospital, Miami, Fla.

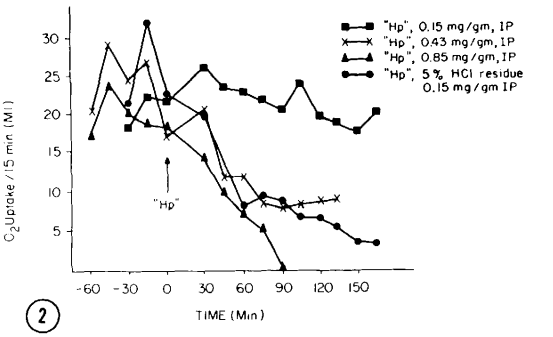
‡ Results summarized in ref. 1 and 2 will be reported in detail elsewhere.

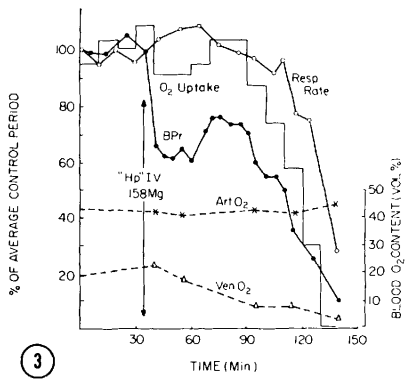
spectively. Usual anaerobic precautions were observed for blood sample collection. Blood oxygen content values were determined by the Van Slyke method and corrected for the lowered hemoglobin levels observed following porphyrin injection. Previously cross-matched blood was administered to 2 rabbits to replace the successive 5 ml portions of blood removed.

Results. 1. Mouse studies: Seventy-eight mice were administered various doses of "Hp" intraperitoneally in a total volume of 0.5 ml and observed for survival. Twenty-seven other mice were injected similarly for individual oxygen uptake studies. Preinjection values averaged 1.0 ml oxygen uptake per minute per mouse. Injection of vehicle alone did not significantly alter this value. Results are summarized in Table I. The observed MLD_{100} was 0.55 mg/gram body weight, while the approximate LD_{50} was 0.3 mg/gram. The latter dose depressed oxygen uptake by an average of 50%.

The MLD_{100} of "Hp" from different







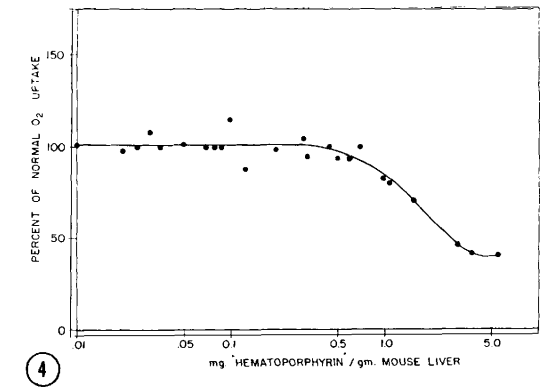


FIG. 1. Respirometer for study of oxygen uptake in mice. Volume of oil (saturated with O_2) required to replace oxygen consumed and thus equalize the 2 fluid levels in manometer is measured periodically.

FIG. 2. Effect of "hematoporphyrin" (IP) on total body oxygen uptake in mice.

FIG. 3. Cardiovascular and respiratory effects of a lethal dose of "hematoporphyrin" administered IV to a rabbit. Average control values: systolic blood pressure (BPr), 100 mm Hg; oxygen uptake, 23 ml/min; resp. rate, 62/min.

FIG. 4. Effect of "hematoporphyrin" on oxygen uptake by mouse liver homogenate. Data plotted for relative cumulative uptake of oxygen during first 30 min after addition of porphyrin or vehicle to homogenate system. Conditions: A 10% liver homogenate was prepared with 0.25 M sucrose. One ml was added to a Warburg vessel and the following were then added: 0.5 ml of 0.25 M. Sucrose: 0.8 ml of a solution containing 0.04 M $MgCl_2$, 0.2 M potassium phosphate buffer pH 6.5, 0.04 M (Na_2K_2) ATP, and 0.01 M potassium malate; and 0.2 ml of 0.1 M sodium pyruvate. Center well contained 0.2 ml of 5 N KOH on filter paper to absorb CO_2, 0.5 ml of "hematoporphyrin" or of vehicle alone were added from the side arm after a 30 min control period of oxygen uptake measurements. Measurements were continued at 10 min intervals for 100 min.

TABLE I. Mortality and O_2 Uptake of Mice Administered "Hematoporphyrin" Intraperitoneally.

Dose "Hp" (mg/g)	Mortality		O_2 Uptake	
	# Mice	% Died	# Mice	% of Control*
.20	5	0	2	75
.30	11	55	6	50
.40	31	81	11	37
.50	15	93	6	30
.55	5	100	—	—
.60	11	100	2	23

* Oxygen uptake 3 hr post-injection of porphyrin compared to pre-injection values.

sources was found to vary widely, from 0.2 to 0.7 mg/gram body weight, depending both on the source and on route of administration of the porphyrin. Results following administration of 2 preparations by each of 3 different routes are summarized in Table II.

Following intraperitoneal injection of a MLD_{100} the mice invariably died during the first 24-48 hours. At the LD_{50} level mortality was greater between 48 and 96 hours.

Progressive changes in oxygen uptake following intraperitoneal administration of porphyrin are shown in Fig. 2. Results with "Hp" are compared with those of the more toxic 5% HCl residue, the latter fraction containing porphyrins extracted from ethyl acetate by 5% HCl after prior removal of hematoporphyrin by 1% HCl. Its LD_{50} was approximately 0.1 mg per gram body weight.

Decreased peripheral blood flow was observed in mice administered an MLD_{100} of "hematoporphyrin" as measured by the cut tail technique.

2. Rabbit studies: In each of the 4 rabbits studied the most significant changes observed were (a) fall in blood pressure, (b) fall in venous oxygen content and increased arterial-venous oxygen difference, and (c) fall in hemoglobin concentration.

TABLE II. Relation of Route of Administration to Mortality in Mice Administered Preparations of "Hematoporphyrin" from Two Different Sources.

Route of administration	LD_{50} (mg/g)		MLD_{100} (mg/g)	
	1	2	1	2
Intravenous	.35	.25	.60	.30
Intraperitoneal	.30	.10	.55	.20
Subcutaneous	.50	.15	.70	.20

1 = "Hp" from Mann Research Laboratories.

2 = "Hp" from Merck Sharpe & Dohme. (It should be noted that subsequent purer material from this source was comparable to the Mann product in toxicity.)

Results for one of the rabbits are illustrated in Fig. 3. The approximately parallel behavior of systolic blood pressure and oxygen uptake is evident, with a precipitous fall in both during the second hour following intravenous injection of 158 mg of "Hp" (69 mg/kg). It is also noteworthy that during the early post-injection period there is often a transient increase in blood pressure and oxygen uptake and decrease in the arterial-venous oxygen difference.

Hemoglobin concentration fell progressively from 10.6 g% to 6.8 g%. Arterial and venous carbon dioxide content remained constant at 58 and 62 volumes per cent respectively. There was no significant change in rectal temperature.

3. Tissue homogenate studies: The effect of "Hp" on *in vitro* respiration of mouse liver homogenate was studied at pH 6.5 to 8.9. After preliminary study of oxygen uptake for a period of 30 minutes, varying amounts of "Hp" or of vehicle alone were added from the side arm of Warburg flasks. In the different studies the final concentration of added "Hp" varied from 0.02 to 5.0 mg per gram of liver. During the first hour O_2 uptake was essentially constant over the entire range of pH studied in the absence of added porphyrin, and no significant change was observed with any amount of added porphyrin at pH 7.0 to 8.9. However, a considerable decrease in oxygen uptake was found in homogenates incubated at pH 6.5-6.6 following addition of 1.0 mg "Hp" per gram of liver (5.5×10^{-5}M) or more. (This concentration is comparable to that found in the liver during the first few hours following injection of a LD_{50} dose of "HP".) Results and further details are given in Fig. 4.

Discussion. A wide variety of physiological effects of porphyrins has been described. Many of these involving the endocrine, cutaneous, cardiovascular, and neuromuscular systems have been reviewed by Vanotti(4). Except for the details regarding the photodynamic effects of porphyrins, however, findings have often been inconsistent and contradic-

tory. Since most of the studies have employed "hematoporphyrin," the finding that variable amounts of side products are always present in supplies of this material must certainly be a major explanation of these inconsistencies. In our own experience, for example, some samples produce marked lowering of blood sugar in rabbits, others do not; some localize well in tumors, others do not; some increase radiosensitivity of transplanted mouse tumors, others do not(2). These effects, like those described here, appear to differ quantitatively and perhaps qualitatively depending on the relative amounts of the other porphyrins included in the "Hp" mixture.

Of major interest in the present study is the finding of hypotension, decreased blood flow, decreased total body oxygen uptake, and decreased venous O_2 content with apparent production of tissue hypoxia in animals administered large doses of "Hp." Those few porphyrins which considerably enhance radiosensitivity of transplanted tumors in mice do so only with small doses of porphyrin; at larger doses this effect is lost and then replaced by a protective effect(2). Since hypoxia itself protects against radiation effect, this may hold the key to understanding and controlling the undesirable dose-dependent effect of the porphyrins.

The exact nature of the changes observed has not been established. It may be noted, however, that porphyrins in general are related to oxygen metabolism, either as oxygen carriers or respiratory enzymes. The studies of Granick and Gilder(5) on the antagonistic effect of "hematoporphyrin" and protoporphyrin as well as those of Silverstein(6) and others on inhibition of oxidative enzymes by porphyrin suggest the possibility of a direct cellular effect on respiration. The observed fall in venous oxygen content, however, is similar to that associated with decreased blood flow and is quite unlike the elevated venous oxygen pattern seen with respiratory poisons such as cyanide. It is also consistent with the observation that in mice administered an MLD_{100} of "hematoporphyrin" there is almost no blood flow from the cut tail.

The finding that "Hp" inhibited respiration of liver homogenate at pH 6.5 to 6.6 but not at pH 7.0 to 8.9 may also be of interest in relation to the tumor studies. The pH of tumors tends to be lower than that of normal tissues and may be further reduced to pH 6.5 or less following glucose administration (7). There are no known published studies comparing the metabolic effects of porphyrins on tumor and non-tumor tissues *in vitro* or *in vivo* under these conditions.

Some of these relationships are discussed further in the companion report(3).

Summary. The toxicity of several preparations of "hematoporphyrin" has been studied in female ZBC mice and in rabbits. The MLD_{100} in mice ranged from 0.2 to 0.7 mg per gram body weight depending on source of the porphyrin and route of administration. The intraperitoneal route was generally most toxic. A marked fall in total body oxygen uptake associated with severe hypotension and decreased blood flow occurred at lethal or near-lethal dose levels. *In vitro* measurement of oxygen uptake by mouse liver homogenates showed no effect at porphyrin concentrations up to 3×10^{-4}M (5 mg/gram of liver in a 10% liver homogenate) at pH 7.4 to 8.9 but 50 per cent or more depression at pH 6.5 to 6.6. Associated phenomena and some implications of these results in relation to studies on the modification of radiosensitivity by porphyrins are considered.

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

J. MODELL† AND S. SCHWARTZ

Department of Medicine, University of Minnesota School of Medicine, Minneapolis

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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† Present address: Dept. of Anesthesiology, Univ. of Miami School of Med., Jackson Memorial Hospital, Miami, Fla.