STUDIES UPON THE PHYSIOLOGICAL ACTION OF HEMATOPORPHYRIN.

By HANS SMETANA.

(From the Department of Pathology, Peking Union Medical College, Peking, China.)

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INTRODUCTION.

The behavior of animals having non-pigmented skin when injected with a suitable dose of hematoporphyrin, is well known. If such an animal is kept in the dark, no striking difference in behavior occurs from that observed in a normal, uninjected animal kept under the same conditions. On the other hand, if an animal having received such a dose of hematoporphyrin is exposed to sunlight or to strong, well cooled artificial light it soon becomes restless, violently scratches the nose, ears and other parts of the body, frequently jumps high into the air and shows livid discoloration of the skin; the respiration is rapid and the pulse rate increased. Following a short period of such activity the animal grows very weak and listless, gradually falls into coma, develops slow, very deep respiration, a slow pulse, and soon dies. This physiological state has been called hematoporphyrin shock.

The anatomical changes taking place in an animal subjected to this experiment are quite inadequate to explain the cause of death, or to account for the nature of the rôle which either light or hematoporphyrin plays in the process.

Soon after exposure to light is begun, edema of the skin appears, in white mice first becoming manifest in areas unprotected by hair, such as the eyelids, ears and tail. Rigor mortis occurs rapidly. The blood is very dark and clots slowly after death. Hemorrhages may be found in the internal organs, and in some cases blood is present in the lumen of the intestine. The mesenteric vessels, which after death are always found to be distended with blood, occasionally show marked infiltration of their walls with leucocytes, but this finding is frequently absent in even the most severe cases. Slight infiltration of leucocytes in the edematous skin may occur.

Animals injected with such a fatal, sensitizing dose of hematoporphyrin and then exposed to diffused daylight do not develop the acute symptoms of acute hematoporphyrin shock just described. They show signs of irritation, and after repeated injections with hematoporphyrin and exposures to light, their ears become necrotic and drop off. Following a period of cachexia, such animals die, but show no significant or consistent anatomical changes. Alterations in the central nervous system have been described by Eppinger and Arnstein (1).

Shock is never produced by exposure of only a small portion of the body to sunlight after injection of hematoporphyrin. The skin of the exposed area, however, becomes edematous and red, and after a few days shows necrosis. A deep ulcer develops at this point which heals slowly. Microscopically one finds thrombosis of the small vessels in the margin of the ulcer, with necrosis and cellular infiltration of its surface.

After intravenous, intraperitoneal or subcutaneous injection, hematoporphyrin may soon be found in the stomach and intestine, bile and urine. The blood serum at this time contains only a faint trace of hematoporphyrin.

We thus see that hematoporphyrin in suitable doses, *per se*, produces no marked effect when injected into animals. When, however, such animals are exposed to sunlight soon after injection of the drug, very characteristic and striking symptoms appear which rapidly progress to a state of coma soon ending in death.

Anatomical studies performed upon such animals reveal no adequate explanation of the mechanism by which the combined action of light and hematoporphyrin produces death. Comparatively little is known of the physiological state of the body produced by the combined action of hematoporphyrin and light. As the result of a series of experiments performed *in vitro*, Gaffron (2) has concluded that hematoporphyrin acts as a catalyzer which, under the influence of light, causes acceleration of oxidation and accordingly rapid consumption of oxygen.

The following experiments have been performed in the attempt to elucidate further the physiological characteristics of the condition which has been described as hematoporphyrin shock, as well as to test the validity of the theory concerning the nature of the action of hematoporphyrin proposed by Gaffron (2).

All of the hematoporphyrin used in these experiments was prepared by the well known method of Nencki (3).

Effect of Feeding Hematoporphyrin.

An attempt to sensitize white mice to light by feeding hematoporphyrin was ineffective.

Four mice were fed upon bread only, soaked in a weak solution of hematoporphyrin and dried. Two of these animals were kept constantly exposed to diffused daylight in the laboratory for 2 months, while the other two were kept in the dark. Upon numerous occasions during this time all four mice were exposed to direct bright sunlight for several hours. No changes in the skin, general appearance or behavior were noted in any of the four animals. Hematoporphyrin was constantly present in the stools but was never found in the urine.

The Relationship of the Cutaneous Pigment to the Effect of Hematoporphyrin and Light.

Since it is well known that exposure to light after injection of hematoporphyrin affects only white animals or those whose skin is slightly pigmented, it was of interest to determine whether this protection is of purely physical nature or in some other way associated with the pigment.

Albino and slightly pigmented rats and mice were stained a dark blue-black color with Verhoeff's hematoxylin and injected, along with unstained controls, with doses of hematoporphyrin adequate to cause death of the controls upon exposure to sunlight. A number of both groups of animals were exposed to sunlight and the others kept either in the dark or in the subdued light of the laboratory. The unstained controls promptly developed hematoporphyrin shock when exposed to sunlight, while the darkly stained animals, which had just received the same amounts of hematoporphyrin as had the controls, were completely protected from the sun's rays. Repeated exposure of these animals to sunlight produced no untoward effects.

It was clear from this experiment that the same degree of protection offered by cutaneous pigment can be produced by staining the skin of non-pigmented animals. Accordingly, one seems justified in assuming that the natural pigment of the skin plays only a physical rôle in the protection of animals injected with hematoporphyrin from light.

None of the stained or control animals kept in the dark or in subdued light showed any changes in their general behavior. Frozen sections of the skin of these two groups of animals, as well as those exposed to sunlight and normal animals, were made and treated with dioxyphenylalanine (Dopa reaction) to see if any alteration in the cutaneous pigment had been produced by any of these procedures (4). No changes of this nature could be demonstrated.

Sensitizing Effect of the Blood of Animals in Hematoporphyrin Shock.

It has so far been impossible to demonstrate any sensitizing substance in the blood of animals during the period of hematoporphyrin shock.

White mice were injected intraperitoneally with 1.5 cc., or intravenously with ½ cc. each, of the heart's blood of guinea pigs drawn at the height of hematoporphyrin shock and kept in diffused daylight. No difference could be seen between

the behavior and general appearance of these animals and mice similarly injected with an equal amount of normal guinea pig blood and kept under the same experimental conditions. All of these mice were very ill for a few days following the injection, but recovered.

Since injection of blood from another species was obviously undesirable, the sensitizing effect of the blood of animals in hematoporphyrin shock was tested in another way.

A parabiosis of two young white rats of the same litter and sex was performed. 6 weeks after the operation, when both rats were in good condition, one of these animals was injected intravenously with trypan blue. The skin of the non-injected rat, as well as that of the injected one, soon became colored by the dye, thus proving the existence of adequate vascular connections between the circulations of the two animals. The first rat was then injected subcutaneously with 4 cc. of a 1 per cent alkaline solution of hematoporphyrin and exposed to bright sunlight, while the second rat was protected from the rays of the sun by a wooden box which was cut out on one side so as not to obstruct the blood vessels connecting the circulations of the two animals. The injected and exposed rat soon showed the characteristic symptoms of sensitization to light, passed into hematoporphyrin shock and died. The other animal, protected from the light, showed no changes in appearance or behavior. Just before the death of the first rat, the two animals were separated and the wound in the side of the unexposed rat closed. This animal lived and appeared to be entirely normal during the several months it was kept in the laboratory. No pathological changes were found at autopsy.

Exposure of the Peritoneum to Light after Injection of Hematoporphyrin.

Two cats of about equal size were used for this experiment. One animal was given subcutaneously 15 cc. of 1 per cent alkaline hematoporphyrin and shortly afterwards, under ether anesthesia, the peritoneal cavities of both animals were opened widely, and exposed to bright sunlight. The peritoneum was kept moist with normal saline, and the rest of the body well protected from the light by towels. After 3 hours exposure of the peritoneal cavity to sunlight, the cat which had been injected with hematoporphyrin died. At this time the peritoneal cavity of the control animal, which was still in good condition, was closed and the anesthesia stopped. This latter animal quickly recovered.

This single experiment suggests that exposure of any other large surface of the body to sunlight after the injection of hematoporphyrin has the same effect as the exposure of non-pigmented skin. It was observed that the serosa of the intestines of the injected cat became very pale during the exposure to sunlight, while that of the normal animal showed marked hyperemia.

Effect upon the Blood Pressure of the Exposure to Strong Light of (a) Circulating Blood of Normal Animals, (b) Circulating Blood after Injection of Hematoporphyrin.

(*a*)

Reed (5), experimenting with normal dogs, reports a marked fall of blood pressure, sometimes reaching shock level, upon exposure to strong arclight of the blood alone. He interposed a glass tube, 3 mm. in diameter, between the divided

TABLE I.

(*a*) *Exposure to Arclight and Sunlight of Circulating Blood of Normal Animals.*

Type of experiment	Animal	Hours of exposure	Exposure	Mean blood pressure		Fall in blood pressure	Remarks
				Beginning of experiment	End of experiment		
			min.	*mm. Hg*	*mm. Hg*	*mm. Hg*	
Cannula interposed in femoral artery or connecting femoral artery and vein exposed to arclight	Dog 1	4.40 p.m.– 7.10 p.m.	150	115	80	35	
	Dog 2	4.16 p.m.– 5.30 p.m.	74	135	100	35	
	Dog 5	3.20 p.m.– 4.40 p.m.	80	140	110	30	
	Cat 101	3.15 p.m.– 5.00 p.m.	105	140	60	80	
Cannula connecting femoral artery and vein exposed to sunlight	Dog 8	11.16 a.m.–12.45 p.m.	89	130	130	0	Jan.
	Cat 21	11.16 a.m.– 1.15 p.m.	119	110	60	50	May
	Cat 24	10.52 a.m.–12.51 p.m.	119	110	60	50	May (spiral cannula)
	Cat 27	10.11 a.m.–11.40 a.m.	89	150	100	50	May (spiral cannula)

ends of the carotid artery, and exposed the blood flowing through this tube to a beam of light supplied by a powerful carbon arc. In some cases the tube was cleaned repeatedly during the experiment, while in others coagulation of the blood was prevented by injection of heparin. Reed remarks that the result was somewhat modified at times by the use of heparin.

In repeating Reed's experiments in slightly modified form upon cats and dogs such a great fall in blood pressure as he reports has not been observed.

In our experiments a quartz glass tube was either interposed between the cut ends of the divided femoral artery of the anesthetized animal, or it was used to connect the femoral artery and vein. Coagulation of the blood was prevented by injection of heparin. The blood of cats and dogs while flowing through such a tube was exposed, in some cases, to a cooled beam of light composed of the rays

TABLE II.

(b) Exposure to Arclight and Sunlight of Circulating Blood after Injection of Hematoporphyrin.

Type of experiment	Animal	Hours of exposure	Exposure	Mean blood pressure		Fall in blood pressure	Amount of hematoporphyrin injected
				Beginning of experiment	End of experiment		
			min.	*mm. Hg*	*mm. Hg*	*mm. Hg*	
Cannula femoral artery and vein. Intraven. inj. hematoporphyrin. Cannula exposed to arclight	Cat 20 (1,800 gm.)	4.00 p.m.–5.50 p.m.	110	135	30	105	Intraperiton. inj. 20 cc. 0.5% hematoporphyrin
	Cat 105 (2,100 gm.)	11.20 a.m.–1.30 p.m.	130	100	65	35	Intraven. inj. 15 cc. 0.8% hematoporphyrin
	Dog 2 (5,200 gm.)	5.30 p.m.–7.10 p.m.	100	100	90	10	Intraven. inj. 15 cc. 4% eosin
Cannula femoral artery and vein. Intraven. inj. hematoporphyrin. Cannula exposed to sunlight	Cat 22 (1,900 gm.)	11.30 a.m.–1.55 p.m.	145	120	80	40	May, 20 cc. 0.5% hematoporphyrin
	Cat 23 (1,850 gm.)	11.30 a.m.–2.00 p.m.	150	120	65	55	May, 20 cc. 0.5% hematoporphyrin (spiral cannula)

from a 1000 watt carbon arc, and in other instances to sunlight. The mean blood pressure was recorded directly from the carotid artery.

As seen in Table I, there occurred a considerable fall in mean blood pressure in all cases except Dog 8, in which no fall in pressure followed

89 minutes exposure of the circulating blood to direct sunlight. In no instance, however, did the mean blood pressure fall below 60 mm. Hg. In the experiments performed upon Cats 24 and 27, a spiral cannula, 3 mm. in diameter, was used as an exposure chamber for the blood instead of the simple glass tube employed in the other experiments. In this way, a much larger quantity of blood was constantly exposed.

From this group of experiments, therefore, it does not seem that exposure of the circulating blood alone to strong light produces any strikingly greater fall of arterial pressure than might be expected to take place following approximately 2 hours anesthesia carried out in the ordinary light of the laboratory.

(*b*)

In this group of animals, the preceding experiment was repeated immediately after the injection of a sufficient dose of hematoporphyrin to sensitize the animals to sunlight should the entire body be exposed. In all cases the bodies of the animals, however, were protected from the light, and only the blood circulating through the glass tube allowed to come in contact with the rays of light.

The results of this experiment, as summarized in Table II, show that in only one instance, Cat 20, did a greater fall of arterial pressure occur than took place in the normal animals receiving no injection of hematoporphyrin. In one case eosin, which has sensitizing properties similar to those of hematoporphyrin, was injected. None of these animals died during the experiment, but were killed when exposure of the blood to light was discontinued.

Changes Produced in Vitro by Light upon Blood Containing Hematoporphyrin.

Though no differences could be elicited between normal animals and those injected with a sensitizing dose of hematoporphyrin by exposing the blood flowing through a glass tube to strong light, it was not felt that this experiment adequately ruled out the possibility that the striking changes occurring in sensitized animals upon exposure of the body surface to sunlight, might be associated with changes taking place within the blood itself. Accordingly a series of experi-

ments was performed to test the direct effect of hematoporphyrin and sunlight upon blood *in vitro*.

Blood was obtained from both cats and dogs either by venipuncture or directly from the heart and mixed with a small amount of heparin or potassium oxalate to prevent clotting. Each sample was divided into three equal parts. Hematoporphyrin (0.002 gm. to 5 cc. of blood) dissolved in normal saline and a few drops of N/10 sodium hydroxide were added to two of the tubes, while to the third tube only similar amounts of saline and N/10 sodium hydroxide were added. Tubes 1 and 3 were exposed either to sunlight or to the well cooled rays of the 1000 watt carbon arc. Tube 2 was always kept in the dark. The blood in Tube 1, containing hematoporphyrin, invariably changed to a very dark, brownish red color upon

TABLE III.

Changes in the Oxygen Capacity and Content of Blood Produced in Vitro by Addition of Hematoporphyrin and Exposure to Sunlight.

Tube 1		Tube 2		Tube 3	
Blood + hematoporphyrin Exposed to sunlight		Blood + hematoporphyrin Kept in dark		Blood + hematoporphyrin Exposed to sunlight	
O_2 capacity	O_2 content	O_2 capacity	O_2 content	O_2 capacity	O_2 content
vol. %	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*
18.1	6.5	18.0	15.7	18.1	16.5
19.0	6.2	17.0	12.0	17.1	16.8
17.0	2.0	17.5	12.0	17.5	12.0
	3.4		13.0		13.4

exposure to light, the change becoming more marked as the exposure was prolonged. The blood in Tube 2, containing an equal amount of hematoporphyrin, but kept in the dark, showed no change of color. Tube 3, which contained no hematoporphyrin, did not show any change of color even after prolonged exposure to bright sunlight.

It is clear, therefore, that blood to which hematoporphyrin has been added changes to a dark, brownish red color upon exposure to sunlight, and that no such change occurs if such blood is kept in the dark. In the attempt to learn the reason for the striking change of color produced by sunlight in blood containing hematoporphyrin, the oxygen and carbon dioxide contents, as well the oxygen capacity of the three varieties of blood mentioned above were determined by the methods of Van Slyke (Tables III and IV).

From these two tables it will be seen that a marked diminution of oxygen content and increase of carbon dioxide content regularly occurred in the blood of Tube 1, which contained hematoporphyrin and had assumed a dark brownish red color upon exposure to sunlight. The oxygen and carbon dioxide contents of Tube 2, containing blood and hematoporphyrin, which was kept in the dark for the same period that Tube 1 was exposed to sunlight, remained essentially the same as Tube 3, which contained only normal blood without hematoporphyrin, but which was exposed to sunlight along with Tube 1. No changes in color were ever noted in Tubes 2 and 3. The oxygen capacity of all three samples of blood was always approximately the same. Exposure

TABLE IV.

Changes in the Carbon Dioxide Content of Blood Produced in Vitro by Addition of Hematoporphyrin and Exposure to Sunlight.

Tube 1	Tube 2	Tube 3
Blood + hematoporphyrin Exposed to sunlight	Blood + hematoporphyrin Kept in dark	Blood + hematoporphyrin Exposed to sunlight
Carbon dioxide content	Carbon dioxide content	Carbon dioxide content
vol. %	*vol. %*	*vol. %*
32.1	20.8	19.0
28.8	20.8	17.8
25.0	16.8	18.8

of the surface of the blood in the test-tube to the air did not appear to influence the character of the changes regularly observed to occur in Tube 1. This was demonstrated by covering the surface of the blood in all three tubes with a layer of paraffin oil in about half of the experiments.

Causes for Change in Color of Blood Containing Hematoporphyrin When Exposed to Sunlight.

It seems clear that the diminution of oxygen and increase of carbon dioxide content, which has been shown to take place in blood containing hematoporphyrin when exposed to light *in vitro*, are the principal factors which account for the alteration of color of such blood taking place under these conditions. Such qualitative changes, however,

could hardly be held responsible for the distinct brownish tinge which invariably accompanies the darkening in color of such blood.

It was suspected that the brownish color might be due to the presence of methemoglobin, but this was quickly shown to be untrue by repeated spectroscopic examinations of different samples of blood in which the characteristic color changes had occurred. In no instance could a trace of methemoglobin be detected.

TABLE V.

Resistance of New Blood Cells.

Per cent of NaCl in the test-tubes....	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.44	0.46	0.48	0.50
Test-Tubes A	+++	+++	+++	+++	+++	+++	++	++	++	+	+	±
containing	+++	+++	+++	+++	++	++	++	+	+	+	±	±
blood and hem-	+++	+++	+++	++	++	++	++	+	±	±	±	−
atoporphyrin exposed to light												
Test-Tubes B con-	+++	+++	+++	+++	++	++	++	+	+	±	−	−
taining blood	+++	+++	+++	++	++	+	+	+	−	−	−	−
and hemato-	+++	+++	++	++	++	+	+	+	±	−	−	−
porphyrin kept in dark												
Test-Tubes C con-	+++	+++	+++	++	++	++	+	+	±	−	−	−
taining blood	+++	+++	+++	++	++	++	+	+	±	−	−	−
without hema-	+++	+++	++	++	+	+	+	+	±	±	−	−
toporphyrin exposed to light												

+++ complete hemolysis, ++ marked hemolysis, + hemolysis, ± trace of hemolysis.

After centrifugalization, the plasma of Tubes 1, 2 and 3 showed marked differences in color. That of Tube 1 was always very dark red, of Tube 2 a much lighter red, while the plasma of Tube 3 showed only a reddish tinge. Upon pipetting off the plasma and washing the red corpuscles with normal saline solution, the three tubes were again centrifugalized. The saline of Tube 1 was dark red, that of Tube 2 reddish tinged, while that of Tube 3 was colorless. Upon repeating this process upon the same three samples of red corpuscles,

the saline of Tube 1 was still red though of somewhat lighter shade than before, while the saline of both Tubes 2 and 3 remained colorless.

These findings suggested that an increase in fragility of the red blood corpuscles occurred in blood containing hematoporphyrin when exposed to sunlight *in vitro*. Fragility tests were accordingly performed upon the blood of three animals (guinea pigs) to test this hypothesis. Each sample of blood was divided into three equal parts and subjected to the same procedures as outlined above for Tubes 1, 2 and 3. The results of this experiment are summarized in Table V.

As will be seen from Table V, no appreciable difference in resistance could be demonstrated in the red corpuscles of the blood which contained hematoporphyrin and had been kept in the dark, and those of normal blood to which no hematoporphyrin had been added and which had been exposed to sunlight. On the other hand, a distinct increase in fragility of the red cells appeared to have taken place in the blood to which hematoporphyrin had been added before exposure to sunlight. It is at present not possible to state the mechanism by which this fragility of red blood cells is brought about by the combined action of hematoporphyrin and sunlight, but it seems clear that this phenomenon plays an important rôle in the staining of the plasma which occurs regularly under the above mentioned conditions.

The brownish tint observed after exposure to sunlight in blood containing hematoporphyrin is probably due largely to changes in the hematoporphyrin itself. When an alkaline solution of this substance is exposed to sunlight in a test-tube, its bright red color soon changes to a dark, brownish red. This change occurs either in an open test-tube, or when the solution is covered with a layer of paraffin oil to protect it from the air. Such a brownish red solution shows a slight spectroscopic difference from alkaline hematoporphyrin kept in the dark, an extra line appearing in the spectrum between *b* and *c*. Its physiological action, however, remains unaltered. The minimal lethal dose for mice, 0.015 gm., and the amount necessary to fatally sensitize mice to sunlight, 0.005 gm., are the same as for alkaline hematoporphyrin in which this change of color has not occurred.

The Blood Pressure in Hematoporphyrin Shock.

The blood pressure in the carotid artery of cats, under ether anesthesia, was recorded before, during and after the injection of sensitiz-

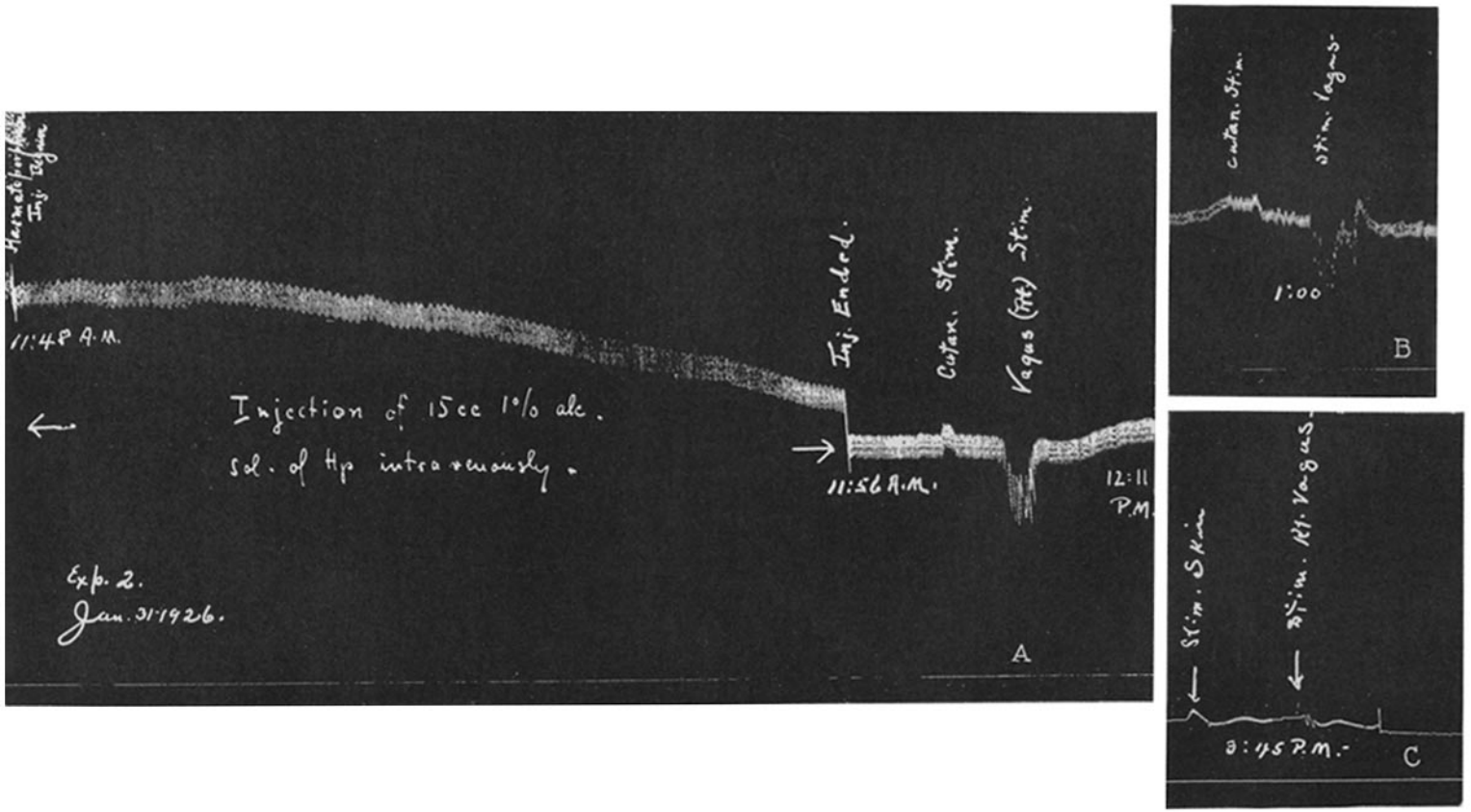

CHART 1. The blood pressure after injection of hematoporphyrin and exposure of the body to sunlight.

ing doses of hematoporphyrin and exposure of the animals to direct sunlight. The crystalline hematoporphyrin was always dissolved in 1 to 2 per cent sodium bicarbonate, and 0.1 gm. of hematoporphyrin per kilo of body weight injected intravenously or subcutaneously.

TABLE VI.

Effect of Exposure to Sunlight upon the Arterial Blood Pressure of Cats Sensitized with Hematoporphyrin.

Animal No.	Date	Length of time of exposure	Exposure	Mean blood pressure		Fall in blood pressure	Remarks
				Before exposure to light	Just before death		
			min.	*mm. Hg*	*mm. Hg*	*mm. Hg*	
Cat 1 (15,508 gm.)	Jan. 17	12.30 p.m.– 1.58 p.m.	88	150	40	110	Intraven. inj. 15 cc. 1% hematoporphyrin
Cat 2 (2,650 gm.)	Jan. 31	11.48 a.m.– 1.06 p.m.	78	120	50	70	Intraven. inj. 20 cc. 1% hematoporphyrin
Cat 3 (2,065 gm.)	June 16	10.10 a.m.–10.45 a.m.	35	120	40	80	Subcut. inj. 25 cc. 1% hematoporphyrin
Cat 25 (1,800 gm.)	May 10	11.00 a.m.–12.15 p.m.	75	120	40	80	Subcut. inj. 25 cc. 0.8% hematoporphyrin

After exposure of such an animal to direct sunlight, the respiration soon becomes more rapid, and the blood pressure falls steadily. After 30 to 60 minutes the respiration has generally become very deep, slow, irregular and labored. At this point the animal is in sufficiently deep coma for the anesthetic to be removed, and after a widely variable length of time (a few minutes to an hour or more) dies. The mean arterial pressure falls rapidly for a time, until the animal is in coma,

and then continues to fall gradually, reaching 40 or 50 mm. just before death (Table VI). The rapidity of the appearance of coma and the fall of blood pressure seems to be directly proportional to the intensity of light and the amount of hematoporphyrin injected. Respiration and heart beat cease simultaneously. Typical blood pressure responses to vagal and cutaneous stimuli were obtained throughout the experiment (Chart 1).

TABLE VII.

Effect Produced upon the Carbon Dioxide-Combining Power, Oxygen Content and Oxygen Capacity of the Blood of Guinea Pigs by Exposure of the Animals to Sunlight after Injection of Hematoporphyrin.

Normal animal exposed to sunlight			Animal injected with hematoporphyrin and kept in dark			Animal injected with hematoporphyrin and exposed to sunlight		
Carbon dioxide-combining power	Oxygen content	Oxygen capacity	Carbon dioxide-combining power	Oxygen content	Oxygen capacity	Carbon dioxide-combining power	Oxygen content	Oxygen capacity
vol. %	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*
47.5	14.0	19.5	48.5	12.9	18.5	16.6	6.5	19.2
40.9	12.6	18.7	37.5	11.5	18.9	19.5	10.5	19.6
44.7	11.8	18.8	38.5	13.7	19.1	18.3	6.9	18.8
54.1						20.2	9.0	
						19.8		

Chemical Changes in the Blood of Animals during Hematoporphyrin Shock.

The following experiments, which were performed upon guinea pigs, were repetitions *in vivo* of the experiments performed *in vitro* upon mixtures of blood and hematoporphyrin already described.

Three animals were used in each experiment. Two of them were injected with a fatal, sensitizing dose of hematoporphyrin. One of these was kept in the dark, while the other, along with the third guinea pig, which had received no hematoporphyrin, was exposed to direct sunlight. Neither the injected animal kept in the dark, nor the normal guinea pig which was exposed to sunlight, showed any alterations in behavior, but the animal which had received hematoporphyrin and was then exposed to sunlight developed the characteristic symptoms of shock as produced under these conditions, and soon died.

Just before the death of this last animal, blood was drawn in a syringe from the right ventricles of the hearts of all three guinea pigs, and immediately placed

under paraffin oil without coming in contact with the air, coagulation being prevented by potassium oxalate mixed with the blood in the syringe. The carbon dioxide-combining power, as well as the oxygen content and oxygen capacity of each of these samples of blood, was determined.

In Table VII, it will be seen that there was little or no difference in the carbon dioxide-combining power of the blood of normal guinea pigs exposed to sunlight and that of animals injected with a fatal sensitizing dose of hematoporphyrin and kept in the dark. Those animals which had received an equal dose of hematoporphyrin and then been exposed to sunlight showed a marked reduction in carbon dioxide-combining power of the blood. No significant difference was found in the

TABLE VIII.

Effect Produced upon the Non-Protein Nitrogen, Creatinine and Sugar of the Blood of Guinea Pigs by Exposure of the Animals to Sunlight after Injection of Hematoporphyrin.

Normal animal exposed to light			Animal injected with hematoporphyrin and kept in dark			Animal injected with hematoporphyrin and exposed to light		
Creatinine	Sugar	N.P.N.	Creatinine	Sugar	N.P.N.	Creatinine	Sugar	N.P.N.
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
2.0	200	27	2.5	160	33	2.3	250	26
1.5	181	33	2.5	166	31	3.0	285	31
1.3	154	32	2.7	285	37.5	2.5	166	35
2.1	222	31.6				2.3	106	42.8
3.0	200	46.1				1.5		
		37.6						

oxygen content of the bloods of normal guinea pigs exposed to sunlight, and those injected with hematoporphyrin and kept in the dark. Guinea pigs injected with hematoporphyrin and exposed to sunlight showed a marked diminution of oxygen in their blood. No change could be demonstrated in the oxygen capacity of blood from these three types of animals.

The non-protein nitrogen, sugar and creatinine of the blood taken from these three groups of animals were determined, but no significant alterations of the amounts of these substances under any of the experimental conditions were found. The results of these studies are summarized in Table VIII.

The red corpuscles and the leucocytes were counted in cats before the injection of hematoporphyrin, and during all stages of shock experimentally produced by this substance. The results of these counts have corroborated the observations of other workers. The red blood cells, which averaged about 8,800,000 per c.mm. in normal cats, showed no significant change during the entire period, which began with the injection of hematoporphyrin and ended with death. The leucocytes, which averaged about 6,000 per c.mm. in normal animals, gradually increased during the experiment to 15,500 per c. mm., and, just before death, rather quickly fell to 4,500 per c.mm. The body temperature was always observed to fall gradually during the development of hematoporphyrin shock. In spite of the fact that the heat of the sunlight was often very intense, the rectal temperature often reached 28–30°C. just before death.

SUMMARY.

The results of these observations may be briefly summarized as follows:

Feeding of hematoporphyrin to white mice over long periods of time produced no apparent changes in these animals and had no effect upon their sensitivity to light.

Albino and slightly pigmented mice and rats injected with hematoporphyrin were protected from the rays of the sun by staining them a blue-black color with Verhoeff's hematoxylin. The dioxyphenylalanine (Dopa) reaction revealed no changes in the cutaneous pigment of animals injected with hematoporphyrin and exposed to sunlight, kept in the dark or diffused daylight. It was therefore assumed that the natural pigment of the skin plays only a physical rôle in protecting animals injected with hematoporphyrin from sunlight.

Exposure to sunlight of only the intestine and mesentery of a cat under ether anesthesia, which had been injected with hematoporphyrin, was followed by death of the animal.

Repeated injections into white mice of large amounts of blood from guinea pigs in hematoporphyrin shock failed to produce symptoms of hematoporphyrin shock. In a parabiosis experiment, one of a pair of white rats promptly developed characteristic symptoms and died

when injected with hematoporphyrin and exposed to sunlight, while the other animal, which was protected from light, but whose circulation had been demonstrated to connect freely with that of its partner, showed no changes during the entire procedure. It has, therefore, been impossible, so far, to demonstrate any substance present in the blood of animals in hematoporphyrin shock which is capable of reproducing this condition in other animals when introduced into the circulation.

Injection of hematoporphyrin followed by exposure of the entire animal to sunlight has been found to produce physiological changes in cats similar to those observed in traumatic shock. There promptly occurred a rapid fall of blood pressure to a very low level and marked lowering of body temperature. The venous blood was found to be poor in oxygen, rich in carbon dioxide and to show low carbon dioxide-combining power. The respiration, which first was accelerated, later on became deep and irregular. The reflexes and typical blood pressure responses to cutaneous and vagal stimulation could always be obtained until death.

Marked diminution of oxygen and increase of carbon dioxide content were found to occur in mixtures of blood and hematoporphyrin exposed *in vitro* to sunlight. These changes in the blood, identical with those occurring *in vivo* during hematoporphyrin shock, support Gaffron's views regarding the effect produced by the combined action of hematoporphyrin and light, but do not further elucidate the nature of the manner in which such alterations take place.

Unsuccessful attempts were made to produce, in both cats and dogs, physiological changes similar to those observed in hematoporphyrin shock by exposing only the blood flowing through a quartz glass cannula, connecting the femoral artery and vein, to strong arclight and sunlight. In another series of animals, which were first injected with hematoporphyrin, exposure of the circulating blood alone to arclight or sunlight did not produce hematoporphyrin shock, although the blood pressure did fall to an unusually low level in one instance.

No changes were found to occur in the amount of non-protein nitrogen, sugar or creatinine of the blood of animals in hematoporphyrin shock.

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