THE ACTION OF VISIBLE LIGHT ON THE HÆMATOPORPHYRIN SENSITISED ORGANS. By J. V. SUPNIEWSKI.

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THE biological importance of short waves of light is commonly recognised, but careful investigations have not shown any significant action of visible light on the biological functions of the animal.

It was found, however, that many fluorescent dyes sensitise biological reactions to the action of visible light. The fluorescence of these substances consists of much shorter waves than the waves of light which they are absorbing.

Hausmann(1, 2, 3, 4, 5) showed that hæmatoporphyrin, which is a fluorescent substance, can sensitise not only protozoa and blood cells but also whole organisms of higher animals to the action of light. Mice injected with hæmatoporphyrin become very sensitive to light. The exposure of such animals to the action of the sun causes acute skin inflammation followed by death. Only meso- and homo-porphyrins show any hæmatoporphyrin-like action; other derivatives of hæmoglobin or chlorophyll are without light-sensitising properties(6). Hæmatoporphyrin alone is not changed chemically when exposed to the action of light(7).

Fischer(8) found that the urine and fæces of light-sensitive men such as those suffering from certain skin diseases (variola vacciniforme) contain hæmatoporphyrin-like bodies. Meyer-Betz(7) showed that man can be sensitised to the action of light by injections of hæmatoporphyrin. The symptoms of this artificial light-hypersensitivity were similar to those described in human disease.

Hæmatoporphyrin seems to be a general sensitiser of photo-chemical reaction. Photographic plates can be sensitised to the action of yellow light by means of hæmatoporphyrin solution. Sunlight causes a destruction of fibrinogen in the presence of hæmatoporphyrin solution. The systemic action of visible light on the organs of hæmatoporphyrin-sensitised animals has not been studied previously. The consideration of this problem forms the substance of the present paper.

The hæmatoporphyrin used in the following experiments was prepared according to Willstätter's modification of the Nencki-Zaloski method. A 1 p.c. solution of this substance in a corresponding quantity of sodium hydrate was used for the experiments. The *p*H of this solution varied between 7·9 and 8·2.

The source of light in my experiments was gas-filled electric bulbs either 200, 150 or 50 candle-power.

The action of light on sensitised smooth muscles.

Adler(9) observed that plain muscle contracts when exposed to the action of ultra-violet light. Identical results were obtained on exposing the phloxine or eosine-sensitised muscle to the action of visible light. In my experiments the organs were sensitised by means of hæmatoporphyrin solution.

Rabbit intestine. Visible light of 150 c.p. at 10 cm. distance causes no change in the rhythm or tonus of the isolated rabbit intestine. A solution of 1/10,000 hæmatoporphyrin in Ringer has also no effect on the movements of the intestine. A stronger solution such as 1/1000 causes a decrease of the intestinal automatic movements.

The exposure of a piece of intestine suspended in a 1/50,000 hæmatoporphyrin solution to the action of light, 150 c.p. at 10 cm., causes a marked decrease of automatic movements and an increase of tonus. This intestine when exposed to the action of light for 15 minutes shows

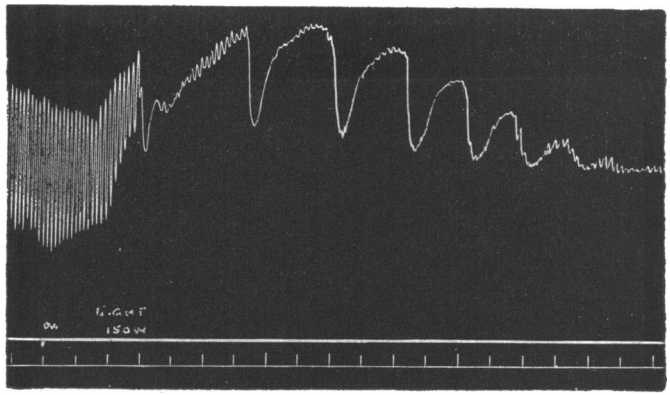

Fig. 1. Isolated rabbit's intestine. Light 150 w. electric bulb, nil 5 c.c. hæmatoporphyrin added to bath to make 1 in 150,000 = nil. The figure shows the subsequent action of the same light.

complete inhibition of movements, and the muscle no longer responds to the action of histamine or barium salts.

The production of intestinal tonus depends on the intensity of the light: very feeble light causes a slow and incomplete contraction. Light of intensity 40 c.p. at 24 cm. has no effect on the hæmatoporphyrin-sensitised intestine. Different coloured lights also exhibit a different action. Red or blue light is without effect, green causes a slight contraction, while yellow rays produce the maximum contraction. The light which passes through 1/500 hæmatoporphyrin solution causes no contraction of sensitised intestine. These experiments indicate that the most active rays are absorbed by hæmatoporphyrin solution. This is in agreement with the results of Fabre and Simmonet(10) obtained with hæmatoporphyrin-sensitised erythrocytes. These experiments require controlling photometrically.

The contraction of the intestine is not dependent on the strength of the hæmatoporphyrin solution: the minimal concentration of hæmatoporphyrin which causes a marked contraction of intestine is 3/500,000. Greater strengths than 1 in 20,000 do not increase the action of the solution and cause no change in the latent period or in the intestinal tonus. Much stronger solutions of this substance are necessary to cause contraction of an intestine when suspended in rabbit's serum.

The intestine absorbs hæmatoporphyrin from the solutions. A piece of intestine which has been left for 15 minutes in 1/5000 hæmatoporphyrin solution and washed for 20 minutes in Ringer is very sensitive to the action of light.

Rabbit, uterus. Rat, uterus. A virgin rabbit's uterus suspended in

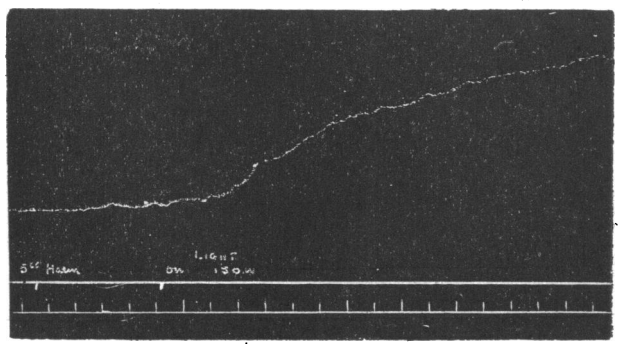

Fig. 2. Isolated rabbit's uterus (virgin). Light = 0. Hæmatoporphyrin 1 in 10,000 = 0. Light after hæmatoporphyrin shown in figure. Time = 30 sec.

1/10,000 hæmatoporphyrin solution, on being exposed to the action of light (150 c.p. 10 cm.) contracts gradually. Under similar conditions the contraction of the non-virgin uterus is more rapid.

The isolated uterus of the rat sensitised by the presence of 1/50,000 hæmatoporphyrin in Ringer's solution contracts gradually in the light of 150 c.p. 10 cm. power, and the automatic movements slowly cease.

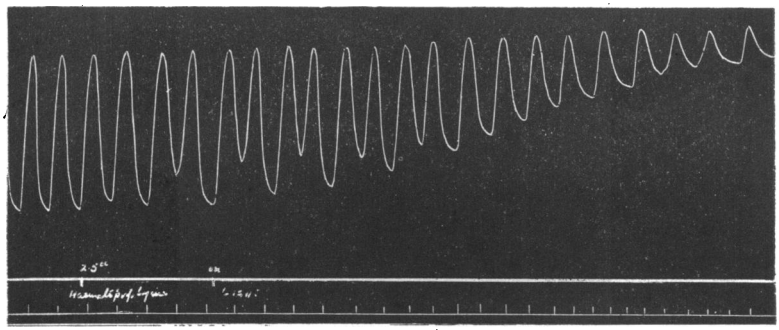

Fig. 3. Rat's uterus (virgin). Light = 0. Hæmatoporphyrin 1 in 50,000 = 0. The figure shows the subsequent action of light. Time = 30 sec.

The following isolated and hæmatoporphyrin-sensitised tissues are sent into contraction by the action of visible light: spleen, aorta and bronchi of the rabbit; and the aorta, pulmonary artery and vena cava of the sheep.

Frog, stomach and bladder. Visible light (150 c.p. 10 cm.) causes a gradual contraction of the isolated bladder or the stomach of the frog sensitised in a solution of 1/50,000 hæmatoporphyrin. This contraction is much slower and more gradual than the plain muscle of warm-blooded animals. The automatic movements of these organs of the frog soon cease under the influence of light, and a long exposure such as 45 minutes causes death.

The action of light on the organs in situ.

Cat, intestine. The intestinal movements of a cat anæsthetised by chloralose were recorded by the rubber balloon method. Visible light of 150 c.p. at 30 cm. power passing through a glass window into the peritoneal cavity has no effect on the tonus or peristalsis of intestines. Also the intravenous injection of 0·03 grm./kg. of hæmatoporphyrin in the dark causes only a slight decrease of peristalsis. Subsequent exposure of these intestines to the action of light causes a local vaso-constriction associated with a rise of blood-pressure and depression of intestinal

movements. The tonus of the intestine is increased. The oncometer tracing showed that the intestinal blood vessels are constricted. Further exposure of the intestine to the action of light causes a lowering of blood-pressure and death of the animal in three to five hours.

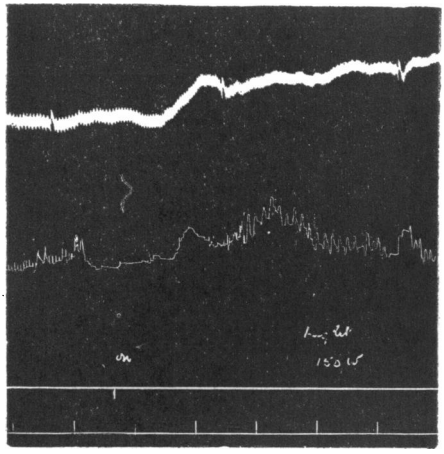

Fig. 4. Cat. Chloralose. Balloon in intestine which can be seen by a glass window in abdomen. Light is without action on intestinal movement. Injection of 2 c.c. hæmatoporphyrin given intravenously = slight decrease of peristalsis. The figure shows the subsequent action of light. Time = 30 sec.

If the uterus of an anæsthetised cat is arranged so that its contractions can be registered, then neither the exposure of the organ to light (150 c.p. 40 cm.) nor the intravenous injection in the dark of 0·02 grm./kg. of hæmatoporphyrin has any effect on the contractions: but the exposure of the hæmatoporphyrin-sensitised uterus to light causes distinct contraction of the uterus.

Similarly the spleen volume of the rabbit is not changed by an exposure to light of 150 c.p. 30 cm.: also the intravenous injection of 0·03 grm./kg. of hæmatoporphyrin in the dark causes only a slight contraction of this organ following the lowering of blood-pressure, but the subsequent exposure of the spleen to the action of light, 150 c.p. 30 cm., causes marked constriction.

If a rat is injected subcutaneously with 0·1 grm./kg. of hæmatoporphyrin and 45 minutes later the uterus and intestines are placed in Ringer's solution so as to record contractions, they respond to visible light, though not in a marked degree.

The action of light on skeletal muscle of the frog.

The isolated gastrocnemius-sciatic nerve-muscle preparation of the frog is not sensitive to the action of visible light. A solution of hæmatoporphyrin of 1/50,000 causes a slight decrease of nervous irritability. The exposure of the hæmatoporphyrin-sensitised preparation to the action of light (150 c.p. at 25 cm.) causes slight contraction of the muscle and a slow decrease of the contractibility to stimulation.

After 30 minutes the muscle is dead. Visible light seems to have no action on the irritability of hæmatoporphyrin-sensitised motor nerve.

The action of light on cardiac muscle. Amsler and Pick(11) showed that moderate exposure to visible light of the hæmatoporphyrin-perfused heart of the frog causes a heart block. In my experiments isolated rabbit's hearts perfused with 1/3000 hæmatoporphyrin solution in Ringer's fluid were used.

Light alone (150 c.p. at 10 cm.) has no effect on the isolated heart of the rabbit. Hæmatoporphyrin solution causes an increase of the heart's activity. The exposure to light of hæmatoporphyrin-perfused heart causes a constriction of the coronaries, slowing of heart rate and a decrease of heart contraction. Ten to fifteen minutes later the heart is dead. Less intensive light (150 c.p. at 22 cm.) causes irregularity of heart beat.

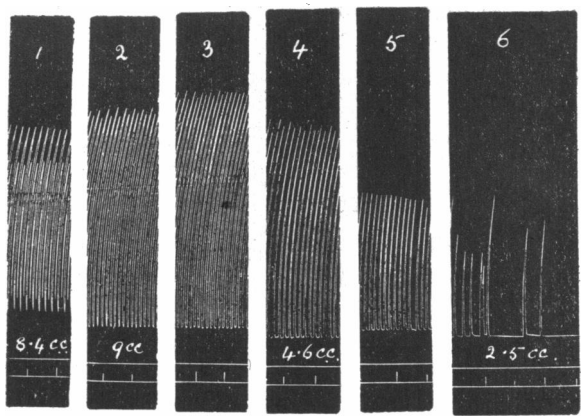

Fig. 5. Isolated rabbit's heart perfused with Ringer. 1. Normal. 2. After hæmatoporphyrin 1 in 3000. 3. Two minutes after application of light arranged 11 cm. from heart. 4. Five minutes after light. 5. Seven minutes after light. 6. Ten minutes after light. The lower figures are the rate of flow through the coronaries per minute. Time = 30 sec.

Further experiments were made on the cat's heart *in situ*. Cats were anæsthetised with urethane and the heart placed in a glass oncometer

so that it could be exposed to direct action of light. Such light shows no change in the cardiac beat. The intravenous injection of 0·02 grm./kg. of hæmatoporphyrin solution causes a transient weakening of heart muscle, lowering of the blood-pressure and a slight increase of the heart contractions. But when the heart is exposed to the action of light (150 c.p. at 30 cm.) it shows a gradual but constant decrease of contraction, an increase of the volume through weakening of cardiac muscle: this effect is associated with a steady fall of blood-pressure. The cat dies 2½ hours later.

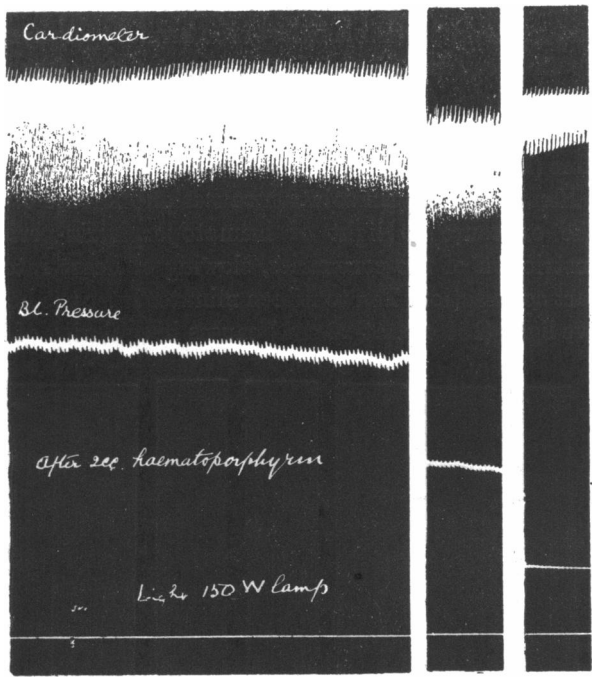

Fig. 6. Cat. A.C.E. urethane. The heart was enclosed in a glass cardiometer. Shows the action of light after hæmatoporphyrin. The separate tracings represent intervals of ½ hour and 2 hours.

The action on the blood-pressure and on the respiration.

The intravenous injection of 0·03 grm./kg. of hæmatoporphyrin causes a transient lowering of the blood-pressure. A shaved albino rabbit injected intravenously with 0·03 grm./kg. of hæmatoporphyrin and exposed to the action of light (200 c.p. 15 cm.) during a short time shows

all the symptoms of skin inflammation associated with a constant lowering of blood-pressure, diminution of the respiratory movements, decrease of blood cells and of hæmoglobin concentration. Three and a half hours later the animal dies.

Discussion.

Strong concentrations of hæmatoporphyrin cause a depression of the cellular function. The peristaltic movements are depressed, plain muscle relaxed, and the heart muscle weakened. The weakening of the heart is the cause of lowering of the blood-pressure which follows the intravenous injection of this drug. Dilute solutions of hæmatoporphyrin have no effect on the biological functions of tissues, but they sensitise them to the action of visible light. Visible light exerts on tissues an ultra-violet-like action. A prolonged exposure of hæmatoporphyrin-sensitised organs to the action of light kills them: the erythrocytes are hæmolysed, spermatozoa, protozoa, ciliated epithelium of the frog are killed, plain muscles are contracted and killed, skeletal muscles or cardiac muscles are relaxed and killed.

The light which is absorbed by hæmatoporphyrin solution is the most active. Hæmatoporphyrin-sensitised animals are killed by the action of visible light with the symptoms of skin inflammation or anæmia Kichiya(6), lowering of the blood-pressure and depression of the respiratory movements. It is difficult to judge which of these effects predominates and which is the cause of death.

Conclusions.

1. Hæmatoporphyrin sensitises isolated organs of animals to visible light. Yellow light seems to be most active.

2. Visible light causes *in vitro* and *in situ* a contraction of plain muscles that have been sensitised by hæmatoporphyrin. This contraction is dependent on the intensity of the light.

3. Visible light kills hæmatoporphyrin-sensitised hearts of warm-blooded animals.

4. Visible light kills hæmatoporphyrin-sensitised animals with symptoms of skin inflammation, anæmia, lowering of blood-pressure and depression of the respiration.

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