THE PHOTODYNAMIC ACTION OF EOSIN AND ERYTHROSIN UPON SNAKE VENOM.

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The study of the destructive action of photodynamic substances upon cells, toxins, ferments, and other chemical bodies, has opened up an interesting field of biological and chemical research. This paper will deal with the effects of two active photodynamic chemicals—eosin and erythrosin—upon the toxic principles of venom.

The starting-point of this study is the complex nature of snake venom, with its several independent toxic principles The effects of these principles are clearly demonstrable by biological tests *in vitro* and in the animal body. Since the action of snake venom has been extensively and profitably studied, the properties of the principles have been fairly established.[1] As regards their chief activities these principles can be divided into the following: neurotoxines, hæmolysins, hæmagglutinins, cytotoxines, hæmorrhagin, thrombokinase, and precipitin.[2] They differ not only in physiological action and chemical composition, but they show widely different labilities.

[1] Mitchell and Reichert, *Smithsonian Contributions*, 1886, No. 647; Cunningham, *Scientific Memoirs by the Medical Officers of the Army of India*, 1895, Part IX, and 1898, Part XI; Kanthack, *Report of Medical Officers of Local Government Board*, London, 1895–1896; Stephens and Myers, *Journ. of Path. and Bact.*, 1899–1900, xi, 415; Stephens, *idem*, p. 273; Flexner and Noguchi, *Journ. of Exp. Med.*, 1902, vi, 277; *Journ. of Path. and Bact.*, 1905, x, 111; *Univ. of Penna. Med. Bulletin*, 1902, xv, 360; Noguchi, *Journ. of Exp. Med.* 1905, vii, 191; Mitchell and Stewart, *Mem. of National Acad. of Sciences*, 1898, viii; Kyes, *Berl. klin. Woch.*, 1903, xl, 956, 982; Kyes and Sachs, *idem*, 1903, xl, 21, 57, 82; Lamb, *Indian Med. Gazette*, 1901, xxxvi, 443; Martin, C. J., *Proc. Roy. Soc. of N. S. Wales*, 1892, xxvi, 240.

[2] This is the globulin and hæmoglobin precipitating body described by me. *Journ. of Exp. Med.*, 1905, vii, 191.

If the variation in resistance of these principles to moist heat is taken the order will be about as follows: neurotoxin resists brief boiling; hæmolysin is destroyed at 135° C., hæmagglutinin at 75° to 80°C.; hæmorrhagins, cytolysins, and thrombokinase at 75° C.; precipitin at 96° to 100° C. Now since the venoms of different species and orders of snakes vary according to the prevalence of one or the other class of toxic constituents, the ease with which they succumb to heating depends on the nature of the predominant principles. Hence rattlesnake venom in which hæmorrhagin and possibly other locally acting non-heat resisting poisons are predominant, and daboia venom in which much thrombokinase is contained, are easily diminished in activity by heating to 75° C., at which temperature cobra venom suffers little change in toxicity.

The venoms of the cobra, *Crotalus adamanteus*, and *Daboia Russellii* were exposed to the action of eosin and erythrosin. These anilines were chosen because of their pronounced action upon ferments and toxins.

Action upon the Hæmolytic Principle.—The venoms were employed in the following strengths: daboia, 0.1 per cent.; cobra and rattlesnake, 0.4 per cent. The dyes were used in 0.25 per cent. solution. Four parts of the venom solution were mixed with one part of the aniline solution. Hence they contained 0.05 per cent. of the dye. The mixtures were divided into two parts, one of which was kept in the dark while the other was exposed to sunlight for thirty hours. The controls consisted of venom solutions of the same strength in salt solutions kept under identical conditions as the dye mixture. Dog's blood in 3 per cent. suspension was employed. The total volume in each tube was two cubic centimeters; the reading was made after two hours at 37° C. and overnight at room temperature.

TABLE I.

COBRA HÆMOLYSIN.

Venom solution in c.c.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.1	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.
0.05	"	"	"	Almost C.H.	"	"
0.02	"	"	"	"	"	"
0.01	"	"	"	Moderate H.	"	Almost C.H.
0.005	"	Almost C.H.	Almost C.H.	Slight H.	"	"
0.002	Moderate H.	Moderate H.	Moderate H.	Trace H.	Moderate H.	Moderate H.
0.001	"	"	"	No. H.	"	"
0.0005	"	"	"	"	"	"
0.0002	Slight H.	Slight H.	Slight H.	"	Slight H.	Slight H.
0.0001	Trace H.	Trace H.	Trace H.	"	Trace H.	Trace
0.00005	"	"	"	"	"	"
0.00002	"	"	"	"	"	"
0.00001	"	"	"	"	"	"
0.000005	"	"	"	"	"	"
0.000002	No. H.	No. H.	No. H.	"	No. H.	No. H.
0.000001	"	"	"	"	"	"
Control	"	"	"	"	"	"

A glance at Table I shows that the hæmolysin of cobra venom is reduced in activity very little by exposure to sunlight in the presence of eosin and not at all in the absence of the dye or in the presence of erythrosin. The hæmolysin is, therefore, resistant to the photodynamic action of these two fluorescent bodies. In view of this result it was to be expected that cobra-lecithid prepared by Kyes's method should remain unaffected, a fact which points not only to the relative resistance of the hæmolysin but also to the stability of the compound. A similar series of tests with Crotalus venom gave different results.

The hæmolytic power of Crotalus venom is reduced by almost 200 times the original strength when measured by absence of hæmolysis, and by almost forty times when measured by slight hæmolysis. The dyes are without influence in the dark and exposure to direct sunlight for the period of the experiment does not injure the hæmolysin in the colorless solution.

TABLE II.

CROTALUS HÆMOLYSIN.

Venom solution in c.c.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.2	C.H.	C.H.	C.H.	Almost C.H.	C.H.	Almost C.H.
0.1	"	"	"	Moderate H.	"	Moderate H.
0.05	Almost C.H.	Almost C.H.	Almost C.H.	"	Almost C.H.	"
0.02	Much H.	Much H.	Much H.	Slight H.	Much H.	Slight H.
0.01	Moderate H.	Moderate H.	Moderate H.	Trace H.	Moderate H.	Trace H.
0.005	"	"	"	No. H.	"	"
0.002	"	"	"	"	"	No. H.
0.001	"	"	"	"	"	"
0.0005	Slight H.	Slight H.	Slight H.	"	Slight H.	"
0.0002	"	"	"	"	"	"
0.0001	"	"	"	"	"	"
0.00005	Trace H.	Trace H.	Trace H.	"	Trace H.	"
0.00002	"	"	"	"	"	"
0.00001	No H.	No. H.	No. H.	"	No. H.	"
0.000005	"	"	"	"	"	"
Control	"	"	"	"	"	"

Cobra and Crotalus hæmolysins exhibit, therefore, a wide difference as regards their susceptibility to injury by eosin and erythrosin. This difference is in keeping with the labilities which they show in general. It is, therefore, of interest to ascertain whether the hæmolysin of daboia venom is to be placed with the former or the latter in its reaction to these substances. Table III which gives the results of the test with daboia venom shows it to occupy an intermediate position since its loss of power fluctuates between $\frac{1}{5}$ and $\frac{1}{10}$ according to the level of hæmolysis at which it is measured. With both rattlesnake and daboia venom eosin appears to be a little more active than erythrosin, but the differences are slight.

Effects on the General Toxicity.—The three venoms—cobra, Crotalus, and daboia—used in testing the effect of eosin and erythrosin upon the general venom toxicities sufficed to bring out the relative degrees of resistance of the neurotoxic, hæmorrhagic, and blood-coagulating principles. The toxic action of cobra venom being due mainly to the first principle, any marked reduction in toxicity which might take place would be ascribable to changes in that substance, and the same reasoning is applicable to hæmorrhagin as represented in rattlesnake venom and coagulin in daboia venom.

TABLE III.

DABOIA HÆMOLYSIN.

Venom solution in c. c	Control		Eosin		Erythrosin.	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.3	C.H	C.H.	C.H.	C.H.	C.H.	C.H.
0.2	"	"	"	"	"	"
0.1	"	"	"	"	"	"
0.05	"	"	"	"	"	"
0.02	"	"	"	"	"	"
0.01	"	"	"	Almost C.H.	"	"
0.005	"	"	"	"	"	Almost C.H.
0.002	Almost C.H.	Almost C.H.	Almost C.H.	Moderate H.	Almost C.H.	Much H.
0.001	"	"	"	"	"	Moderate H.
0.0005	"	"	"	Slight H.	"	Slight H.
0.0002	Much H.	Much H.	Moderate H.	"	Much H.	"
0.0001	"	"	"	Trace H.	"	Trace H.
0.00005	Slight H.	Slight H.	Slight H.	No. H.	Slight H.	"
0.00002	"	"	"	"	"	No. H.
0.00001	"	"	Trace H.	"	"	"
0.000005	No. H.	No. H.	No. H.	"	No. H.	"
0.000002	"	"	"	"	"	"
0.000001	"	"	"	"	"	"
Control	"	"	"	"	"	"

The technique of the tests is simple. Stock solutions of venom are mixed with the dyes in solutions. The mixtures are divided into two parts one of which is exposed and the other kept in the dark. A simple solution of the same strength without any admixture is treated in the same manner—one half being exposed and the other placed in a dark chamber. The exposure to sunlight was, as a rule, for thirty hours.

Cobra Venom—0.2 per cent., stock solution 4 parts; 0.25 per cent., eosin or erythosin 1 part. All solutions made in 0.9 per cent. salt. Exposure 30 hours. Toxicity tested in guinea-pigs weighing 350 grams. Intraperitoneal injections.

TABLE IV.

COBRA VENOM AND GUINEA-PIGS.

Venom in grm.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.001	+ 1 h. 25 m.			+1 h. 30 m.		+ 45 m.
0.00075	+ 4 h. 50 m.					
0.0005	+ 2 h. 48 m.			+ 3 h.		+ 4 h. 10 m.
0.0003	+ 3 h. 34 m.					
0.00025	+ 6 h.					
0.0002	+ 12 h.	+ 18 h.	+ 12 h.	+ 24 h.	+ 18 h.	+24 h.
0.00015	+ 18 h.	+ 36 h.	+ 2 days	+ 2 days	+ 38 h.	+40 h.
0.0001	≈	≈	≈	≈	≈	≈
0.000075	≈	≈	≈	≈	≈	≈
0.00005	≈	≈				

+ =death. ≈=Survived.

The conclusion to be drawn from this series of tests is that the two anilines employed are without marked effects in diminishing the toxicity of cobra venom for guinea-pigs. On the other hand, with rabbits, a slight reduction in toxicity after the eosin treatment is apparent.

TABLE V.

COBRA VENOM AND RABBITS.

Rabbit No. 1. Venom 0.002 gram. Death in 20 minutes.
Rabbit No. 2. Venom 0.002 gram + eosin 0.05% (Dark). Death in 20 minutes.
Rabbit No. 3. Venom 0.002 gram + eosin 0.05% (Sunlight). Slightly ill; recovered.
Rabbit No. 4. Venom 0.003 gram + eosin as before (Sunlight). Death in 80 minutes.
Rabbit No. 5. Venom 0.004 gram + eosin as before (Sunlight). Death in 15 minutes.
Rabbit No. 6. Venom 0.008 gram + eosin as before (Sunlight). Death in 60 minutes.

The reduction in toxicity as measured on rabbits probably does not exceed $\frac{1}{3}$ and may be less, since the individual variation in susceptibility of the animals may affect the results. That such a factor operates at times is to be seen by comparing the results with Rabbits Nos. 5 and 6.

Crotalus Venom.—The preponderance of hæmorrhagin and the small quantity of neurotoxin contained in rattlesnake venom make it peculiarily suitable for tests of relative stability of these two classes of bodies. The symptoms and lesions of poisoning by rattlesnake venom can readily be interpreted. Hence any marked reduction in toxicity can be accounted for by the destruction of one or both of the chief toxic principles.

The venom was in 0.4 per cent. and the eosin and erythrosin in 0.25 per cent. solutions. The details of the method were the same as with cobra venom. Guinea-pigs of 250 grams were used for the tests, the injections being made into the peritoneum.

TABLE VI.

CROTALUS VENOM AND GUINEA-PIGS.

Venom in grm.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.03				+ 5 days*		+ 4 days*
0.024				+15 days*		♀
0.018				No symptoms†		No symptoms†
0.012				"		"
0.008				"		"
0.004				"		"
0.001	+ 6 h.‡	+ 5 h. 40 m.‡	+ 8 h.‡	"	+10 h.‡	"
0.0008	+ 8 h.	+12 h.			+15 h.	
0.0006	+ 10 h.	+24 h.	+12 h.		+10 h. 50 m.	
0.0005	+ 24 h.	+ 3 days	+ 24 h.		+24 h.	
0.0004	♀	♀	♀		♀	
0.0003	♀	♀	♀		♀	

\+ = Death.
‡ = Very marked hæmorrhage in the peritoneal cavity, viscera, and muscles.
* = Died of emaciation, no hæmorrhage.
† = Killed with chloroform and the peritoneal cavity examined after 48 hours; no hæmorrhage.
♀ = Survived.

From this table it is seen that it is the hæmorrhagic principle that is destroyed by the dyes after exposure to sunlight. However, the neurotoxin would also appear not to be left wholly intact; but to what change in action of the venom the marasmic condition which developed in certain of the pigs is due is not known. It is well known that many of the bacteria and certain toxins cause in quantities below the lethal dose a state of malnutrition to which the animal may eventually succumb. The remote effects of the modified venom may be compared to this action of bacteria, ricin, and other toxins.

The time limit of thirty hours, while insufficient to reduce markedly the toxicity of cobra venom, is greater than is necessary to reduce considerably the toxicity of rattlesnake venom. A series of tests was made with the latter venom, which was exposed with eosin for eight hours to sunlight. The result with guinea-pigs of 250 grams each was as follows:

0.002 gram. Survived.
0.0032 " "
0.0048 " "
0.0064 " Death after 5 days. Slight hæmorrhage.
0.008 " Sick for ½ day; chloroformed after 36 hours; slight hæmorrhage.

The action of eosin in the light upon Crotalus venom is quite rapid. In eight hours about 9.6m. l. d. and about 16 m. hg. d.[3] of the venom were completely destroyed under the conditions of the experiment.

Daboia Venom.—While this venom is rich in hæmolytic and cytolytic principles its chief immediate peculiarity of action results from the thromobokinase which it contains. Whether neurotoxin is present in any but minimal quantities is still undecided. My experiments with eosin and erythrosin were directed against the blood-coagulating constituent. The venom was used in a proportion of 4 parts of 0.2 per cent. and the dyes in 1 part of 0.25 per cent. solutions. The methods employed were identical with those already given. The time of exposure was thirty hours; rabbits weighing 1500 grams were injected intravenously.

TABLE VII.

DABOIA VENOM AND RABBITS.

Venom solution in c.c.	Control		Eosin		Erythrosin	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
0.03				+ 5 days*		+ 10 days*
0.02				½		½
0.015				+ 5 days*		No sympt.
0.01				½		"
0.008				No sympt.		"
0.006				"		"
0.004				"		
0.003				"		
0.0004	+ 5 min. ×	+ 2 min. ×	+ 6 min. ×		+3 min. ×	
0.0004	+ 4½ min. ×	+ 7 min. ×	+ 5 min. ×		+1½ min. ×	
0.0004	+ 7 min. ×	+ 6 min. ×				

+ = Death. ½ = Survived after some illness.
× = Intravascular thrombosis.
* = Died of marasmus.

Since these experiments (Table VII) show that the clotting principle of daboia venom is completely destroyed by the fluorescent dyes employed in the experiments, and since they also show that by this treatment the general toxicity of the venom may be considerably reduced, a second series of experiments was made to ascertain roughly the rapidity with which the toxicity is diminished by means of eosin. Rabbits of 1500 grams weight were employed.

[3] Minimal hæmorrhagic dose.

TABLE VIII.

DABOIA VENOM AND RABBITS.

Time of exposure	Dose of venom	Results
30 minutes	0.0004 gram.	No symptoms
" "	0.0006 "	+ 5 minutes
" "	0.0008	+ 5 minutes
3 hours	0.0016 "	No symptoms
" "	0.0024 "	+ 7 minutes
6 hours	0.032 "	No symptoms
" "	0.004 "	" "
" "	0.006 "	Sick; survived
8 hours	0.0016 "	No symptoms
" "	0.0032 "	" "
" "	0.0064 "	" "
12 hours	0.008 "	No symptoms
30 hours	0.01 "	No symptoms

These experiments show that the principle causing intravascular clotting of the blood is quickly and readily destroyed by eosin in the light and that 75 m. l. d. of eosinized venom may not contain one m. l. d. of the thrombokinase. The death of the rabbit, which took place after injection of a large amount of the modified poison was due probably to the combined action of the hæmolytic and neurotoxic residues.

Action upon the Red Corpuscle-Protecting Principle.—In a previous publication [4] I described a new property of venom, namely, its capacity to unite with the globulin constituents of blood serum to form an insoluble compound which is precipitated, and with the globin of hæmoglobin which it also renders insoluble in water and weak saline solution. Because of its action upon hæmoglobin strong solutions of cobra venom protect blood corpuscles from water, and even from energetic chemical hæmolysis. Experiments were made with eosin and erythrosin to determine whether this property of cobra venom is destroyed by these agents under the influence of light.

[4] *Jour. of Exper. Med.*, 1905, vii, 191.

The cobra venom was used in 20 per cent. solution, of which equal parts and 0.1 per cent. solutions of the dyes were mixed. One part of the mixture was exposed to sunlight for 12 hours and the other kept in the dark as a control. The solutions for precipitation consisted of (1) washed rat's corpuscles laked in 9 parts of water and centrifugated to remove the stroma, (2) aqueous solutions of Merck's hæmoglobin, and (3) rat's serum diluted with water. The tests were made with 0.8 c.c. of the solutions enumerated and 0.2 c.c. of the venom-dye mixture. The protective action on the corpuscles was tested by adding 0.3 c.c. of washed rat's corpuscles to 0.6 c.c. of the venom-dye mixture. The results are given in Table IX.

TABLE IX.

		Control (no dye)		Eosin.		Erythrosin.	
c. c.	Control (no venom)	20% Cobra venom 1 part Distilled water 1 part.		20% Cobra Venom 1 part 0.1% Eosin 1 part.		20% Cobra venom 1 part 0.1% Erythrosin 1 part.	
		Dark	Exposed	Dark	Exposed	Dark	Exposed
Blood solution 0.8 The fluid tested 0.2	Clear	Voluminous precipitate (18 hours)	Voluminous precipitate (18 hours)	Voluminous precipitate (18 hours)	Voluminous precipitate (18 hours)	Voluminous precipitate (coarser) (18 hours)	Voluminous precipitate (coarser) (18 hours)
2% aqueous solution of hæmoglobin (Merck) 0.8 The fluid tested 0.2	Clear	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)	Voluminous precipitate (instantan.)
Rat's serum 0.2 H_2O 0.6 The fluid tested 0.2	Clear	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)	Marked precipitate (18 hours)
Rat's blood corpuscles (washed thrice) 0.03 The fluid tested 0.6	No hæmolysis	Protected	Protected	Protected	Protected	Protected	Protected

The result is definite. The globulin precipitating and corpuscle-protecting principle of venom does not undergo marked change in sunlight in the presence of eosin and erythrosin after an exposure of 12 hours. If erythrosin in a strength equal to one per cent. of the mixture is present, the venom-hæmoglobin precipitation does not occur, and rat's corpuscles are prevented from protection against hæmolysis. In respect to this action of erythrosin, it should be added that Sacharoff and Sachs state that in solutions of high concentration this dye is itself hæmolytic.

SUMMARY.

Since the hæmolysins of the several venoms respond differently

to photodynamic action, they may be regarded as possessing different chemical constitutions. As regards stability, cobra hæmolysin ranks first, daboia second, and Crotalus third.

The toxicity of all the venoms is more or less diminished by eosin and erythrosin in sunlight. This reduction in toxicity depends upon chemical changes, of more or less profound nature, taking place in certain of the active principles of the venom. The more stabile the predominant active principles the less the reduction in toxicity, and vice versa. Venom-neurotoxins are highly resistant to photodynamic action, venom-hæmolysins are less resistant, while the hæmorrhagin and thrombokinase of Crotalus and daboia venoms exhibit weak powers of resistance to their action. Hence it follows that while cobra venom remained almost unaltered, rattlesnake and daboia venoms were greatly reduced in toxicity when mixed with the fluorescent dyes and exposed to sunlight.

There is an interesting parallel between the action of eosin and erythrosin upon the different venoms and their reactions to other injurious agencies. For example, the hæmolysins of cobra and daboia venoms are more heat resistant than the hæmolysin of Crotalus venom, and the former are less injured by the dyes than the latter. The neurotoxin of the former venoms is also more heat stabile than that of the rattlesnake, and the same relative degree of resistance holds for this substance and the anilines. Just as the hæmorrhagin of rattlesnake venom and the thrombokinase of daboia venom are destroyed by a temperature of 75° C., so are they readily inactivated by the photo dynamic substances employed.

The globulin-precipitating and blood corpuscle-protecting principle of cobra venom is relatively thermostabile and in contradistinction to the immunity-precipitins it is also unaffected by eosin and erythrosin.

This study of the action of photodynamic substances upon snake venoms serves again to bring out the fact of their highly complex nature, and while enlarging somewhat the field in which photodynamic activity is known to operate, it also proves that this form of destructive activity is affected by the same con-

ditions of resistance as confront the action of the usual physical and chemical agents.

THE LITERATURE ON PHOTODYNAMIC ACTION.

In this brief review only the salient facts can be given. The literature on the biological effects of fluorescence has already grown into a considerable bulk. We shall begin with v. Tappeiner's (1) studies on the chinin derivatives. He proved that the toxic property of chinin resided in the chinolin nucleus and that the toxicity of this body was increased when methoxyl and methylene radicals were present in the side chains. V. Tappeiner further endeavored to increase the toxicity of chinolin by bringing about a further condensation with the benzol nucleus. The proof of the correctness of this view was brought by Grethe (2), who found that the dye phosphin exerted intense toxic action upon paramecium. Raab (3) noted that muriate of acridin in a 1 : 1000 dilution kills paramecium instantly; while weaker solutions acted with disturbing irregularity. The cause of this irregularity was finally traced to the influence of light. Thus in a solution of 1: 20,000 in the dark the protozoa are unaffected, while in diffuse sunlight they are destroyed in 60 minutes, and in direct sunlight in 6 minutes. Raab also ascertained that the action of all protoplasmic poisons is not intensified by light: morphine, phenylchinaldin, and strychnin are not modified in their effects by light. Raab discovered that the increased action of light depends upon fluorescence of the chemical agents. Minute studies have now been made of this property. Raab found that when sunlight passes through a fluorescent solution it is robbed of its power to set up fluorescence in a second solution. No intensification of toxic action is produced in the second solution by the filtered light. Fluorescent light itself is without toxic action upon infusoria; to obtain this effect the living organisms must be immersed in the fluorescent fluid.

We owe to v. Tappeiner the term "photodynamic action," which describes the toxic effects produced by fluorescent chemicals in the light. This activity does not depend upon the absorption of light rays, because some of the most active absorbing agents were shown by v. Tappeiner and Jodlbauer (4,5) not to have their toxicity increased by light. All fluorescent substances would seem to be able to exert photodynamic action; but there is much variation in the intensity of action among the different bodies themselves, and a further variation appears according to the substances—living cells, ferments, toxins—upon which the action is exerted. The relation of degree of fluorescence and intensity of photodynamic action is, according to v. Tappeiner and Jodlbauer (6) a reverse one. Strong solutions may act more energetically than weak ones, but a quantitative comparison shows that, as a rule, the weaker concentrations of the fluorescent body are the more active (7). When certain members of a chemical group do not possess fluorescence they tend to be devoid of photodynamic action. Many salts of fluorescein are photodynamically active; but the non-fluorescent salts—tetranitrofluorescein, phenolphthalin, hydrochinonphthalin—are inactive (8).

Besides the degree of fluorescence and concentration of the chemical body, the intensity of the light plays an effective part. Dreyer (9) found that the

infusorian Nassula was killed in 10 seconds under the combined influence of eyrthrosin and Finsen's light, while the Finsen light alone required 9 minutes to produce this effect. Dreyer and Neisser and Halberstaedter (10) viewed the erythrosin merely as a sensitizer and ranked it with neutral red, which they regarded as devoid of fluorescent power. More searching study has brought out the fact of neutral red's possession of feeble fluorescent properties; and v. Tappeiner (11) has been able to show further that sensitization of a photographic plate and photodynamic action are independent powers. Several active sensitizers—bisulphite alizarin blue, diazo black, glycin red, nigrosin, ethylene red—are non-fluorescent and also photodynamically inactive.

In the meantime Straub (12) observed the dissociation of potassium iodide in the light by eosin and chinin, and thus opened the way for the study of photodynamic action as a process of oxidation, proofs of which form of activity were supplied by Edlefsens' (13) studies which were confirmed by Jodlbauer and v. Tappeiner (14,15). The last-named investigators made a comparative study of the oxidative and biologically injurious properties of photodynamic substances and found that a parallel did not exist. The strongest oxidizing body is not the most effective photodynamic agent. Thus aesculin is an energetic oxidizer but has weak photodynamic power, while the biologically active phenosafranin and azocarmin are incapable even of dissociating iodide of potassium. Jodlbauer and v. Tappeiner also found that photodynamic action may exceptionally take place in fluids from which the free oxygen has been removed. Bie (16) believed the bactericidal action of light not to depend on oxidation. This part of the subject clearly calls for further study pending the setting up of conclusions.

A considerable number of unicellular organisms, toxins, ferments, and antitoxins have been studied in relation to photodynamic activity. The infusoria have been especially studied by Raab (17), v. Tappeiner (18), Jodlbauer and v. Tappeiner (19), and Ledoux-Lebard (20). They all show marked sensitiveness to this form of injury. Jacobson (21) has found that the ciliated tracheal epithelium of the frog is injured by acridin especially in the light. Sachs and Sacharoff (22) and Pfeiffer (23) have described the hæmolytic action of certain fluorescent dyes. Some of these are active in the dark the action being, however, intensified by light (24).

Bacteria are subject to photodynamic action as is shown by the observations of Dreyer (25), Raab, Jodlbauer and v. Tappeiner (26), Huber (27), and Bie (28). Huber found that the filtration of the light through ruby glass prevents the injurious action. Fungi are also susceptible to the action of certain of the anilines. Lichtwitz (29) found that the complements but not the hæmolytic immune bodies of normal and immune serums are destroyed by eosin in the light. And according to Fleischmann (30), eosin and light together so modify precipitin that it no longer causes a precipitate with its corresponding precipitable substance, although it is still capable of entering into chemical union with it.

The ferments show varying degrees of susceptibility to photodynamic activity. Diastase, invertin, papayotin (31), trypsin, and chymosin (32) are affected more or less strongly. Eosin and magdala red are among the most active anilines. Diastase is more resistant than invertin and papayotin. Many fluorescent,

photodynamic bodies do not act injuriously upon the ferments. According to Huber (33), rennet is little affected by simple exposure to light, while in the presence of eosin or erythrosin its coagulating action is much delayed. Katalase remains unaffected by any of the fluorescent bodies tested (34).

Bacterial and other toxins succumb to this form of activity (35). Diphtheria toxin is much reduced in power by eosin and light. Other fluorescent anilines are also effective on this poison. V. Tappeiner and Jodlbauer observed that guinea-pigs could be protected from one m. l. d. of diphtheria toxin by previous injection of eosin or methylene blue and daily exposure to the sun. Doses of 3 to 4 m. l. d. were uniformly fatal after this treatment. Huber (36) also found that diphtheria toxin is destroyed by eosin in the light. V. Tappeiner and Jodlbauer noted the modification or destruction of tetanus toxin by certain dyes. They found that after treatment 10 m. l. d. of the toxin did cause local tetanus, while 25 m. l. d. caused fatal tetanus. Huber noted the gradual deterioration of tetanolysin in the light and its rapid destruction in the presence of eosin. Flexner and Noguchi (27) showed that tetanolysin and tetanospasmin are destroyed *in vitro* by eosin, and rats can be protected from fatal tetanus infection by local applications of the dye. Ricin (37) is readily attacked. V. Tappeiner and Jodlbauer found that its agglutinin is totally destroyed by a large number of anilines, while the other toxic constituents are more resistant.

Mammals are not insusceptible to the action of these bodies. Acridin may cause abscesses in rabbits (5), and mice are subject to local necroses if after injection they are exposed to the light. A peculiar effect is the necrosis of the ears of mice, which follows the injection and exposure (38). Exophthalmus has been noted in rats injected with Rose bengale. After injection of eosin, erythrosin, and Rose bengale into the shaved skin of rabbits and exposure to sunlight, an area of necrosis with exfoliation of the tissue develops.

V. Tappeiner and Jesionek (39, 40) have treated with success patients suffering from a variety of parasitic and neoplastic conditions of the skin by applying eosin and exposing the parts to sunlight. Artificial light may be substituted for sunlight and injection into the tissues may sometimes take the place with advantage of superficial application of the dye.

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