

## THE EFFECT OF EOSIN AND ERYTHROSIN UPON THE HÆMOLYTIC POWER OF SAPONIN.

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The chemical action of photodynamic substances is very wide. Not only do they cause alteration of the labile molecules of ferments and toxins, but they are definite and even intense protoplasmic poisons. Their action is, however, not limited to the complex structures and molecules of these bodies, but is capable of being exerted upon much simpler and, chemically speaking, more definite substances.

The more recent investigations of v. Tappeiner,<sup>1</sup> Jodlbauer, Straub,<sup>2</sup> Edlefsen,<sup>3</sup> and Sacharoff and Sachs,<sup>4</sup> all tend to show that fluorescent photodynamic chemicals act by promoting oxidation of the cells, toxins, etc. A wide variety of inorganic and organic well-defined chemical substances was proven to undergo oxidation in the presence of the photodynamic reagents. There were certain exceptions as, for example, dextrose and formaldehyde, which remained unaltered.

In view of these facts, and as a complement to my studies on snake venom, I tested the action of eosin and erythrosin upon the hæmolytic property of saponin. The following tables show that eosin and especially erythrosin act with considerable intensity upon saponin.

The saponin was Merck's, the dyes "eosin rein" and "erythro-

<sup>1</sup> Jodlbauer und v. Tappeiner.—Ueber die Beteiligung des Sauerstoffes bei der Wirkung fluorescierender Stoffe. *Deutsch. Arch. f. klin. Medicin*, 1905, lxxxii, 520.

<sup>2</sup> Straub.—Ueber chemische Vorgänge bei der Einwirkung von Licht auf fluorescierende Substanzen und die Bedeutung dieser Vorgänge für die Giftwirkung. *Münch. med. Woch.*, 1904, li, 1093.

<sup>3</sup> Edlefsen.—Experimenteller Beitrag zum Studium der fluorescierenden Stoffe. *Münch. med. Woch.*, 1904, li, 1585.

<sup>4</sup> Sacharoff und Sachs.—Ueber die hæmolytische Wirkung der photodynamischen Stoffe. *Münch. med. Woch.*, 1905, lii, 299.

sin pur." were Grüber's. Saponin was employed in 0.1 per cent., dissolved in 0.9 per cent., salt solution. The dyes were employed in concentrations of from 0.05 per cent. to 2.5 per cent. The mixtures of saponin and dye were divided into two portions, of which one was kept in the dark, the other being exposed to direct sunlight for twelve hours. Control solutions of saponin were treated in the same manner. Two kinds of corpuscles—from the dog and the rat—were used in 3 per cent. suspension. After four hours in the thermostat at 37° C. and eighteen hours at room temperature the readings were made.

TABLE I.

Saponin 0.1%; fluorescent anilines 0.05%; dog's corpuscles.

Saponin solution 0.1%	Control		Eosin 0.05%		Erythrosin 0.05%	
	Dark	Exposed	Dark	Exposed	Dark	Exposed
1.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.
0.7	"	"	"	"	"	"
0.5	"	"	"	"	"	Much H.
0.4	"	"	"	"	"	Moderate
0.3	"	"	"	"	"	Slight H.
0.2	"	"	"	"	"	None
0.15	"	"	Much H.	Much H.	Much H.	"
0.1	Much H.	Much H.	Moderate	None	Moderate	"
0.07	Slight H.	Slight H.	Trace	"	Trace	"
0.05	None	None	None	"	None	"
0.04	"	"	"	"	"	"
0.03	"	"	"	"	"	"
0.02	"	"	"	"	"	"

TABLE II.

Saponin 0.1%; eosin 0.05%; rat's corpuscles (washed).

Saponin solution	Control		Eosin 0.05%	
	Dark	Exposed	Dark	Exposed
1.	C. H.	C. H.	C. H.	C. H.
0.7	"	"	"	"
0.5	"	"	"	"
0.4	"	"	"	Almost C. H.
0.3	"	"	"	Much H.
0.2	"	"	"	Slight H.
0.15	"	"	"	None
0.1	Almost C. H.	Almost C. H.	Much H.	"
0.07	Much H.	Much H.	Slight H.	"
0.05	Slight H.	Slight H.	None	"
0.04	None	None	"	"
0.03	"	"	"	"
0.02	"	"	"	"

The tables show that saponin alone, in the strength solutions employed, does not deteriorate quickly; and they also show that solutions of this substance have their hæmolytic power

considerably reduced when exposed to sunlight in the presence of minute quantities of eosin and erythrosin, and these fluorescent anilines exert a slightly reducing effect upon this property even in the dark. The maximal loss of hæmolytic power equalled in twelve hours about  $\frac{1}{4}$  to  $\frac{1}{5}$ , and in seventy-two hours  $\frac{1}{4}$  the total strength.

That concentration of the solutions plays a part in the experiment is indicated by the resistance which a one per cent. solution of saponin exhibited in the presence of eosin in strength of 2.5 per cent. The saponin was not in this case perceptibly reduced in hæmolytic strength after twelve hours' exposure to direct sunlight. If, however, a one per cent. saponin solution is mixed with 0.05 per cent. eosin it is reduced to one-fifth its hæmolytic strength after seventy-two hours' direct exposure to sunlight. V. Tappeiner noted that strong solutions of photodynamic substances may be inactive where weaker ones are effective.

The change in constitution which the saponin undergoes in the presence of eosin and erythrosin has not been established. It would seem, from certain indirect experiments which I have made, not to depend upon the usual splitting of the glucoside. While I did not determine the absolute absence of dextrose in the exposed mixtures, yet I can say that a quantity equal to 0.001 per cent. was not present. Beyond this limit the copper tests which I used were not reliable.