THE EFFECT OF EOSIN UPON TETANUS TOXIN AND UPON TETANUS IN RATS AND GUINEA-PIGS.

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The study of the action of certain photodynamic substances upon living cells, ferments, and toxins has brought out highly interesting facts. The literature upon this subject is already large and will be briefly reviewed in the paper of one of us (Noguchi) upon the action of these chemicals upon snake-venom.[1] Jodlbauer and v. Tappeiner [2] studied the action of certain fluorescent substances upon diphtheria and tetanus toxins. They found that when the toxins were mixed with 0.05 per cent. of eosin and exposed for three days to diffuse sunlight their toxicity had greatly diminished. The sample of tetanus toxin thus treated produced in 1 to 10 m. l. d. local tetanus only, while in 25 m. l. d. and over it was fatal. Huber [3] stated that the addition of one per cent. of eosin to tetanus toxin, and an exposure of six hours to the sunlight, suffice to destroy the poison, but if the mixture is kept in the dark the poison is not deprived of its toxicity. The dose used by Huber in testing toxicity was 1 m.l.d. Huber also noted a loss of hæmolytic power.

Our experiments with tetanus toxin were begun last spring and were completed before Huber's paper appeared. They embrace the study, first, of the influence of certain aniline dyes upon tetanolysin; second, the effect of eosin upon tetanospasmin; and, third, the influence of eosin upon the course of experimental tetanus in rats and guinea-pigs.

The Effects of Certain Aniline Dyes upon Tetanolysin.

Ehrlich [4] showed tetanus toxin to be composed of two poisons—the hæmolysin, tetanolysin, and the convulsive body, tetano-

[1] To appear in the next number of this Journal.

[2] Jodlbauer and v. Tappeiner, *Münch. med. Wochenschrift*, 1904, li, 737.

[3] Huber, *Arch. f. Hygiene*, 1905, liv, 53.

[4] Ehrlich, *Berliner klin. Wochenschrift*, 1898, xxxv, 273.

spasmin. Madsen,[5] proved the former to be a highly labile body. Our experiments were made with two samples of the toxin: one a dried ammonium sulphate precipitate of bouillon cultures, and the other a fresh filtrate of bouillon cultures. The first was used as a four per cent. solution; the second in full strength, sterile salt being added to produce isotonicity. The determination of initial hæmotoxicity gave the following:

TABLE I.

	Sample I.	Sample II.
0.2 c.c.	C. H	C. H.
0.15 "	"	"
0.1 "	"	"
0.07 "	"	Almost C. H.
0.05 "	Almost C. H.	"
0.04 "	Moderate H.	Moderate H.
0.03 "	Slight H.	"
0.025 "	Trace H.	Trace H.
0.02 "	"	"
0.015 "	"	"
0.01 "	No H.	No H
0.007 "	"	"
0.005 "	"	"

To two cubic centimeters of each preparation varying amounts of one per cent. eosin in saline were added; the mixtures were kept at 37° C. for one hour and tested against two per cent. washed rabbits' corpuscles. The tubes were incubated four hours at 37° C. and kept eighteen hours at room temperature.

TABLE II.

Tetanolysin 2 c.c.	Sample I.	Sample II.
Eosin 1 % 1.0 c.c.	No H.	No H.
" 0.7 "	"	"
" 0.5 "	"	"
" 0.3 "	"	"
" 0.2 "	Slight H.	Slight H.
" 0.15 "	"	"
" 0.1 "	Moderate H.	Moderate H.
" 0.07 "	Much H.	Much H.
" 0.05 "	Almost C. H.	Almost C. H.
" 0.03 "	C. H.	C. H.
" 0 "	C. H.	C. H.

[5] *Zeitschr. f. Hygiene*, 1900, xxxii, 214.

Two cubic centimeters of tetanolysin (representing about 150 m. h. d.[6]) are completely destroyed by 0.3 cubic centimeter of a one per cent. solution of eosin, in one hour in the dark, at 37° C. In other words, an eosin strength of 0.13 per cent. quickly destroyed the lysin.

Simple exposure of tetanus toxin in test-tubes to sunlight for six hours completely destroyed the lysin; and an exposure for two hours in the presence of 0.03 cubic centimeter eosin solution to two cubic centimeters of the toxin also destroyed the hæmolysin. In the dark this small quantity of eosin has no action upon the lysin. The action of the eosin in the light is very rapid, since after ten minutes' exposure the hæmolytic property may be lost.

A series of experiments were made with Grübler's methylene blue, orange G, orcein, fuchsin, and vesuvin. Berlin blue was also tried. The results may be summarized as follows: In strengths of 0.2 per cent. and above, methylene blue and vesuvin destroy tetanus hæmolysin in one hour. Quantities below 0.1 per cent. are partially destructive only; while 0.04 per cent. no longer checks hæmolysis. Fuchsin, orcein, and fluorescein are also injurious, but their limits of action were not determined. Berlin blue is without effect.

These experiments bring out the fact that aniline dyes, fluorescent ones, chiefly, have an injurious effect on the labile hæmolysin in the dark, although the effect is less than in the light.

The Effect of Eosin upon Tetanospasmin.

The fatal constituent of tetanus toxin is the convulsive agent tetanospasmin, which has an especial affinity for nervous tissues. We tested the action of eosin in the dark as well as in the light upon the convulsive principle. To one cubic centimeter of the toxin (Sample II) were added varying amounts of five per cent. eosin. The mixtures were kept one hour at 37° C. before injection in the right gluteal region into white rats, weighing about 90 grams each. The animals were kept in a room diffusely lighted; the cage was partly shaded. The toxin employed was fatal in four days in a dose of 0.0025 cubic centi-

[6] Minimal hæmolytic dose.

meter, the first symptoms of tetanus appearing in twenty-four hours; 0.0005 cubic centimeter was the minimal tetanic dose, and 0.001 caused strong tetanus but was not fatal. Doses of about 0.4 cubic centimeter caused death in from 24 to 48 hours. Hence one cubic centimeter of the toxin contained about 400 m. l. d. and 2000 m. t. d.[7] for the rats.

Experiments. Rat 1. Toxin 1 c.c. plus 5 % eosin 1 c.c. 1 hour at 37° C in thermostat. Injection of full amount, 10 A.M., Oct. 13. Next day, no tetanus, nor did tetanus develop later. The leg was swollen for several days as result of injection.

Rat 2. Toxin 1 c.c., 5 % eosin 0.5 c.c. Same conditions. Entire quantity injected, Oct. 17. No effect except swelling at injection point.

Rat 3. Toxin 1 c.c., 5 % eosin 0.3 c.c. Same conditions. Injection, Oct. 19, 10 A.M. Oct. 20, slight tetanus; increased next day. Oct. 30, still marked tetanus of injected leg. No general tetanus. Nov. 3, found dead. Lived 15 days.

Rat 4. Toxin 1 c.c., 5 % eosin 0.1 c.c. Same conditions. Oct. 13, 10 A.M., injection. Oct. 14, slight tetanus; next day, marked tetanus. Oct. 19, tetanus increased; dead, Oct. 21. Lived 9 days.

Rat 5. Control. Toxin 1 c.c. Oct. 13, 10 A.M. Next day, marked tetanus. 5 P.M., dead. Lived 29 hours.

These protocols show that eosin in solutions exceeding one per cent. in strength quickly destroys tetanospasmin in the dark, and in 0.6 per cent. solutions reduces greatly the activity of the poison. The chronic nature of the tetanus which the modified poison produces is a striking and interesting occurrence. To what this change is to be attributed—whether to influence of the eosin upon the animal organism or to peculiar changes in the poison itself—has not been determined with accuracy. We shall return to this topic.

The Effect in the Animal Body of Eosin upon Tetanus Toxin.

In this series of tests tetanus toxin and eosin were injected simultaneously but separately into the rat.

Experiments. Rat 1. Oct. 20, 10 A.M. Toxin, 1 c.c. 1 c.c. 5 % eosin immediately injected about the site of introduction of the poison. Oct. 21, slight tetanus, which increased until death on Oct. 24. Lived 4 days.

Rat 2. Same date. Toxin 0.1 c.c. 1 c.c. 5 % eosin immediately injected about toxin site. Oct. 22, no tetanus. Oct. 23, very slight tetanus. Oct. 24, marked tetanus. Increase until death on 7th day.

[7] Minimal tetanic dose

Rat 3. Oct. 27, 11 A.M. Injected toxin, 0.05 c.c., and immediately 1 c.c. 5 % eosin. No tetanus, Oct. 30. On 31st, suspicion of tetanus. Next day, tetanus increased, and then progressive increase until death on 8th day.

Rat 4. Oct. 28, 10 A.M. Toxin, 0.01 c.c. and eosin as before. Oct. 30, suspicion of tetanus; 31st, increased slightly; in next days, further increase. Nov. 10, dead. Lived 13 days.

Rat 5. Oct. 29. Toxin, 0.01 c.c., injected into right leg and 5 % eosin solution 1 c.c. injected into left leg. Oct. 30, right leg not used; Oct. 31, marked tetanus. Nov. 3, dead. Lived 5 days.

In this series of experiments the effect of eosin upon the course of the tetanus consisted in delaying the onset of symptoms and in prolonging the period of intoxication. Since the toxin employed caused, in control animals, in doses of 0.0025 cubic centimeter, tetanic symptoms to appear in twenty-four hours, the delay of symptoms in the eosin-treated animals is at once apparent; while the prolongation of life is also well brought out by the protocols. However, none of the animals of this series was actually saved. And in view of this fact the question arises whether, after all, eosin exerts a destructive influence upon the toxin *in vivo* at all comparable with its effect *in vitro*. Since, in all but one rat, the eosin injection was made about that of the toxin, and since eosin alone in five per cent. solution causes œdema of the tissues, perhaps changes in absorption may account for the delay of onset of the symptoms and fatal effect. But this explanation does not entirely account for the effect observed, as in Rat 5 the injection of the poison in one side and the eosin in the other side of the body was made and yet the fatal result was delayed.

The Influence of Eosin upon the Course of Tetanus in Rats and Guinea-Pigs.

It is recognized that poisoning with tetanus toxin is not an exact equivalent of infection with tetanus bacilli, for while in the first the full fatal dose of poison is at once available for fixation by the tissues, in the second the appearance of the first symptoms of tetanus may not exactly coincide with the presence of a fatal quantity of the poison in the body. If, therefore, eosin is capable of modifying tetanus poison in such a manner as to

diminish its toxicity when the two are kept in contact for a short time at body temperature, even in diffuse light and in the dark, the important question arises whether this action may be exerted in the body to an extent sufficient to rescue infected animals. To imitate natural conditions more closely silk threads carrying tetanus spores but no toxin were introduced beneath the skin of the right thigh. Some of the animals remained untreated; others were treated with eosin in various ways.

Experiments. Rat 1. Control. Inoculated Nov. 1, 10 A.M. Nov. 4, first appearance of tetanus. Nov. 5, marked tetanus. Dead, Nov. 7. Lived 3 days after the appearance of tetanus.

Rat 2. Control. Nov. 5, inoculation as above. Nov. 8, tetanic. Death, Nov. 11. Survived first appearance of symptoms 3 days.

Rat 3. Nov. 1, 10 A.M. Thread introduced as before. Immediately afterwards 1 c.c. 5 % eosin injected in the tissues above the thread. Nov. 2, œdema; leg not used. Nov. 5, no tetanus; small necrosis at site of injection. Dec. 1, no tetanus; animal normal.

Rat 4. Nov. 1, thread introduced. Nov. 3, first appearance of tetanus. 1 c.c. 5 % eosin injected in same leg. Nov. 4–6, tetanus increased. Nov. 11, dead. Lived 9 days after first appearance of symptoms.

Rat 5. Nov. 1, thread introduced in right leg. Nov. 4, slight tetanus. 1 c.c. 5 % eosin injected in left leg. Tetanus increased. Death on Nov. 8. Survived first symptoms 4 days.

Rat 6. Nov. 1, thread introduced. Nov. 2, no tetanus; 0.5 c.c. 5 % eosin injected about the thread. Nov. 3, no tetanus; eosin injection. Nov. 4, no tetanus; injection repeated. Same on Nov. 5, 6, 7, 8. No further injections. Dec. 3, no tetanus. Animal normal.

Rat 7. Nov. 1, thread introduced. 0.2 c.c. 5 % eosin injected every 24 hours as in Rat 6. Nov. 5, slight tetanus, which gradually progressed until Nov. 12, death. Survived first appearance of symptoms 7 days.

Rat 8. Nov. 1, thread introduced. 0.5 c.c. 5 % eosin injected intraperitoneally every 24 hours for four days. Nov. 5, tetanus appeared; Nov. 12, dead. Survived first symptoms 7 days.

This series of experiments indicates that the power of eosin to destroy or modify the tetanic poison in the body, while marked is not absolute. However the eosin is brought into the body of the infected animals, it causes an increase in the period of intoxication and delay in the supervention of death. If the eosin solution is sufficiently concentrated and is brought into relation with the focus of development of the tetanus spores, it is capable not only of modifying the tetanus but of wholly sup-

pressing its development. The manner of this action is not yet wholly worked out, and hence we cannot at present say whether the suppression affects the germination of the spores or merely the detoxication of the poison as it is produced. This part of the subject, as well as still other phases of it, as, for example, the value of combined eosin and anti-toxin treatment of animals poisoned with tetanus toxin and infected with tetanus spores, the effects of still other photodynamic substances, and the influences of the aniline injections upon local absorption, are now being studied. The influence of eosin upon the production of tetanus toxin *in vitro* is also being determined.

A parallel series of experiments to the latter, carried out on the more highly sensitive guinea-pig, will now be given briefly. The pigs weighed 300 grams each.

Experiments. Guinea-pig 1. Control. Nov. 21, thread introduced. Nov. 22, slight tetanus. Nov. 23, marked tetanus; dead in night. Lived 60 hours.

Guinea-pig 2. Control. Nov. 24, 10 A.M. Tetanus spores. Nov. 25, marked tetanus. Nov. 25, 9 P.M., dead. Survived 36 hours. A third control lived 2½ days.

Guinea-pig 3. Nov. 21, 10 A.M. Spores introduced. 1 c.c. 5 % eosin immediately injected into same leg. Nov. 22, leg swollen; no tetanus. Nov. 23 to 28, no tetanus. Animal emaciating; dead of marasmus, Dec. 1. No symptom of tetanus at any time.

Guinea-pig 4. Nov. 21, 10 A.M. Spores; at same time, 1 c.c. 5 % eosin in opposite leg. Nov. 22, marked tetanus. Died during the night of 23d. Survived 60 hours.

Guinea-pig 5. Nov. 21, 10 A.M. Spores. Nov. 22, slight tetanus. 1 c.c. 5 % eosin injected in locality of thread. Nov. 23, no progress of tetanus. Nov. 24, tetanus increased; no further change until Nov. 27. Animal weaker and tetanus increased. Nov. 28, dead. Survived 6 to 7 days.

Guinea-pig 6. Nov. 21, 11 A.M. Same experiment as No. 5, but the eosin injected into opposite leg. Survived 3 days.

Guinea-pig 7. Nov. 21, 10 A.M. Spores. Nov. 22, slight tetanus. 1 c.c. 5 % eosin injected into same leg. Nov. 23, tetanus increased; eosin injection, Nov. 24, eosin injection. Nov. 25, symptoms progressing, eosin injection. Nov. 26, *idem*. Nov. 27, dead. Survived first symptoms 5 days.

This series of experiments shows that even in the highly sensitive guinea-pig tetanic symptoms may be restrained, provided the eosin is brought into close relationship with the developing tetanus poison before it is fixed by the nervous tissues or reaches the general circulation.