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LOCAL IMMUNITY TO TETANUS IN INOCULATED RATS TREATED WITH EOSIN.

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Previous studies having shown the mechanism of the immunity to tetanus which develops in rats treated locally with eosin, my attention was directed by Dr. Flexner to certain indications of the occurrence in the treated animals of a local in contrast to a general immunity to the tetanus bacillus. Our studies[1] had already shown that the spores on silk threads in the healed eosin-treated wounds remain alive for an indefinite period, and the threads removed and transplanted to other rats, or to the opposite side of the body of the same rat, caused fatal tetanus. And yet, the operation for the removal of part of the spore-threads was not followed, in spite of the trauma inflicted and the portion of the spore-thread which remained, by any tentanus in the extremity originally infected. Since it appeared that the conditions were, after the second operation, similar on both sides of the body, or in the two animals, an explanation of the different reactions observed was required. It seemed natural to assume, for the sake of further experiment, that the restricted germination and vegetation which take place in the eosin-treated animals produce a small quantity of tetanus-toxin, which, acting locally upon the tissues, gives rise in them to a degree of immunity to the action of the tetanus poison.[2]

The observations upon which this hypothesis was first based will be given; but it should be stated in advance that the tetanus-bearing threads were introduced subcutaneously in the thigh and not deeply into the tissues. After the healing process is complete, the threads come to lie in a small mass of scar tissue between the superficial

[1] Flexner and Noguchi, *Jour. of Exper. Med.*, 1906, viii, 1.

[2] Noguchi, "The Nature of the Antitetanic Action of Eosin," this number of the JOURNAL.

fascia and the skin. Except for the cicatrix the tissues all return apparently to the normal condition.

Experiment I.—In order to determine whether the spores remained alive in the inoculated rats after the healing process was complete, a portion of a thread was removed thirty days after inoculation and fifteen days after the cessation of the eosin treatment; no trace of the eosin remained at this time in the tissues. The removed portion of the thread was used for preparing cultures and for inoculating rat No. 12. Tetanus bacilli grew in the cultures. Tetanus developed in rat No. 12 on the second day, and death from tetanus took place on the fourth day after inoculation. The treated and operated rat remained well.

Before discussing further the significance of this experiment, a selected series of protocols bearing on this question of local immunity to tetanus will be first given.

Experiment II. Rat No. 13.—Thirty days after inoculation and fifteen days after cessation of eosin treatment, a portion of the healed-in spore-thread was removed from the right thigh. No tetanus followed the trauma, and the wound quickly healed. Six days later, a second portion of the thread was removed and implanted subcutaneously under the skin of the left thigh. Tetanus developed on the left (new) side on the second day, and death occurred on the fourth day after this inoculation. The right (original) hind leg remained entirely free from tetanus.

Experiment III. Rat No. 14.—Eosin treatment. Seventeen days after inoculation the thread was removed from the right thigh; the wound of the second operation was completely healed in seven days, during which period no tetanus developed. July 12, reinoculation of spore-threads beneath the skin of both thighs. July 16, left leg tetanic; right leg free. July 18, tetanus has progressed on left side; right side free. July 19, rat dead. The animal lived three days after the first appearance of tetanus. The right (old inoculated) leg remained free from tetanus until the end.

Experiment IV. Rat No. 15.—Spore-thread inoculation into right leg June 8; eosin treatment until June 20. No tetanus. Spore-thread removed July 28; no tetanus. October 9 (123 days after inoculation, and 111 days after last injection of eosin), 0.00008 c.c. tetanus toxin injected into each thigh (0.0003 = m.l.d. in three days). Tetanus developed in the left leg in forty hours, and advanced rapidly; slight tetanus developed in the right leg after three days, and advanced slowly. Death on seventh day.

Experiment V. Rat No. 16.—Spore-thread inoculation into right leg October 29; eosin rein treatment until November 8. No tetanus. December 1 (33 days after inoculation and 25 days after last injection of eosin) 0.0004 c.c. tetanus toxin injected into each thigh. Tetanus developed in left leg after three days, and advanced rapidly; a mild form of tetanus developed in the right leg and advanced slowly. Both sides became strongly contracted; death on sixteenth day.

Experiment VI. Rat No. 17.—Spore-thread inoculation into right thigh October 19. Eosin gelb injection until November 2. No tetanus developed. December 1 (43 days after inoculation and 29 days after last injection of eosin),

0.0004 c.c. of tetanus toxin injected into each thigh. No tetanus developed in five days. Second injection into each thigh of 0.0004 c.c. toxin. Next day tetanus began in left leg; none in right leg. The tetanus advanced in the left and remained absent from the right side until death, which took place on the ninth day.

Experiment VI brings out clearly, for the first time, the existence of a degree of general immunity to the toxin which is, however, able to off-set a small and limited amount only of the effects of the toxin. The local immunity of the right thigh proved, upon a second injection of the toxin, to be still effective, while the general immunity, through which the left leg had been protected from the consequences of the first injection, was exhausted by it. The next experiment indicates that even a higher degree of general immunity may arise from the local infection.

Experiment VII. Rat No. 18.—Spore-thread inoculation into right thigh; eosin treatment. No tetanus developed. October 9 (123 days after inoculation and 111 days after last injection of easin), 0.00008 c.c. tetanus toxin (0.00008 = m.l.d.) injected into each thigh. No tetanus after three days. October 12, 0.00004 c.c. toxin injected into each leg. October 19, no tetanus developed. The spore-thread from this animal transplanted to another rat produced typical tetanic symptoms, followed by death in two and a half days.

Experiment VII is a stronger confirmation even than experiment VI of the idea that general immunity to tetanus toxin may be developed in the eosin-treated rats. The degree of general immunity is, in almost all the rats, lower than that of the local immunity. Rat No. 18 seems to supply the one exception to what appeared as the rule in this respect. It was now desirable to ascertain by a direct experiment whether the blood of the eosin-treated rats contained a measurable quantity of tetanus antitoxin.

Three series of rats were taken: (1) normal rats as controls; (2) rats which after the spore-thread insertion had been treated with eosin "gelb;" (3) rats which after inoculation had been treated with eosin "rein." The rats were bled from the carotid artery, and the serum collected. On account of the small amount of blood yielded by these animals, the blood of the several animals of each series was collected together. The eosin-treated rats were bled from sixty-four to seventy-four days after the cessation of the eosin treatment. The following tabulation gives the results of the tests. In each instance, the mixtures of serum and tetanus

toxin were kept at 37° C. for half an hour before injection. The injections were made beneath the skin of the thigh.

Serum Injected.	Toxin Injected.	Tetanus Developed.	Result.
Normal 0.3 c.c.	0.006 c.c. (6 m.l.d.)	24 hours.	Death on 3d day.
Normal 0.3 c.c.	0.002 c.c. (2 m.l.d.)	24 hours.	Death on 6th day.
Saline 0.3 c.c.	0.006 c.c. (6 m.l.d.)	24 hours.	Death on 3d day.
Saline 0.3 c.c.	0.002 c.c. (2 m.l.d.)	24 hours.	Death on 5th day.
Eosin gelb "a" 0.3 c.c.	0.006 c.c. (6 m.l.d.)	slightly in 48 hours.	Tetanus increased but did not extend beyond injected leg. 28 days after injection still alive; tetanus gradually disappearing.
Eosin gelb "b" 0.3 c.c.	0.006 c.c. (6 m.l.d.)	slightly in 48 hours.	Tetanus progressed, death on 9th day.
Eosin rein 0.3 c.c.	0.006 c.c. (6 m.l.d.)	slightly in 48 hours.	Death on 10th day.
Eosin rein 0.3 c.c.	0.002 c.c. (2 m.l.d.)	5th day.	Death on 15th day.

The experiments which I have set down in this article seem to me to afford convincing proof of the existence of local immunity to tetanus infection and tetanus intoxication. The observation is, I believe, new and not without interest in its bearing on the theory of the "building sites" of immune bodies in the animal organism. Examinations of the lesion consisting of the scar enclosing the spore-threads have shown it to be superficial, limited to the fascia and adjacent subcutaneous tissue, and to measure a few millimeters in extent only. There are no anatomical changes discoverable which would connect the lesion with the deeper muscles, fascia, nerves, etc.

From the studies made of the spore-threads at different periods during the eosin treatment,[3] a complete idea of the evolutions through which the introduced spores pass has been obtained. A certain small number of the spores germinate and grow into vegetative bacilli, which, after having reached this stage of development, remain as such for a time, or, possibly, pass through a small series of divisions with the production of a few generations of bacilli. The number of divisions is at best few, and the total number of bacilli produced from the spores is relatively small. The vegetative bacilli degenerate within a few days, and the majority of

[3] Noguchi, "The Nature of the Antitetanic Action of Eosin," this number of the JOURNAL.

the spores do not germinate at all, but remaining alive and quiescent, are enclosed in the scar tissue.

Doubtless, there is associated with the low degree of vegetation of the tetanus bacilli toxin production. This toxin, always small in amount, is held chiefly in or near the locality in which it is formed by the surrounding wall of eosin through which it must, in the first place, pass slowly and with difficulty, and which, in the next place, would tend to detoxicate it on its passage. The effect, therefore, of the toxin would be exerted locally, if anywhere, and if any cells capable of being excited to antibody-production exist in the situation in which the toxin finds itself, they, presumably would be stimulated into activity. Should any of the toxin, in a still active state, diffuse beyond the eosin barrier, it would find itself in places from which it could readily pass into the blood and into the internal organs, where it would be exposed to the usual conditions of antibody formation. I conceive that the mechanism of the antibody production in the particular case brought out by these experiments is to be explained somewhat in the manner of this supposition.

The questions immediately arise as to which cells in the local tissues are concerned with the elaboration of the antitoxin, and whether the antoxin as such is stored in these cells for immediate liberation when called forth by the presence of the specific antigen. As regards the first question, no definitive answer can be given. From the circumstances of the experiment, I am led to suppose the connective tissue cells, possibly the endothelium of the lymphatics as well, as the cells yielding the antibody. The extremely superficial character of the lesions must be kept in mind in attempting to fix the parts the cells of which participate actively in the production of this local immunity. But I cannot bring forth proof that the active cells are so strictly limited and so sharply circumscribed as I have indicated. It would, theoretically, seem to be less difficult to supply an answer to the second question. It does not seem to me highly likely that so diffusible a substance as antitoxin in the free state could remain in a state of high concentration through many months in one part of the body at the same time that it existed in very low concentration elsewhere in the body.

On the contrary, I should be more inclined to the view that a large part of the general antitoxic immunity which the rats exhibited may have been derived from the antibody absorbed from its local site of production. The cells of the locally immune tissues have, I assume, undergone a physiological change which endures for many months, at least, enabling them to withstand the injurious effects of tetanus bacilli and tetanus toxin either by means of rapid liberation of antitoxic substances, or by an increased form of resistance to and destruction of poison (Giftfestigkeit) and bacilli with which the liberation of antagonistic antibodies is not necessarily associated. Upon what remarkable changes in function or structure this power depends, we are in total ignorance, and any speculation must, therefore be wholly hypothetical.

The literature on immunity contains, as is well-known, other examples of local immunity. Doubtless in every case, at some period of antibody production, there exists a local immunity which exceeds in degree that which the blood is able to display. This fact must be acknowledged by all who believe antibodies not to be formed chiefly in the blood itself. It will suffice merely to refer to the observations of Pfeiffer and Marx[4] on the organs in which the antibodies to the cholera bacillus are produced; to the experiments of Wassermann and Takaki[5] of the fixation of tetanus toxin by the brain and other organic tissues; to the studies of Wassermann and Citron[6] on the local production by the pleural and peritoneal endothelium of antibodies (bacteriolytic, possibly others also) for the typhoid bacillus; to the interesting experiment of von Dungern[7] on the local production of precipitin for Maja serum in the anterior chamber of the eye; and, finally, to the observations of opthalmologists[8] upon the local immunity developed by the conjunctiva to abrin inoculation, a phenomenon which Römer studied with great care and precision. Theoretically, there is much similarity in all these observations, since they prove that anti-

[4] *Zeit. f. Hygiene und Infektionskrank.*, 1898, xxvii, 272.

[5] *Berl. klin. Woch.*, 1898, xxxv, 5.

[6] *Zeit. f. Hygiene und Infektionskrank.*, 1905, l, 331.

[7] Die Antikorper, Jena, 1903.

[8] Von Hippel, *Archiv f. Ophthalmolog.*, 1883, xxix, 213. Sattler, *Klin. Monatsbl. f. Augenheilkr.*, 1883, xxi, 207. Neisser, *Fortsch. d. Medicine*, 1884, ii, 73. Römer, *Archiv f. Opthalmologie*, 1901, lii, 172.

bodies are produced locally. The gradually increasing number of facts relating to this subject tend to exalt in importance cells which hitherto have been regarded as indifferent in respect to antibody production: namely, the cells of the connective tissues, lymph spaces, lymph vessels and serous cavities. The particular observations which form the basis of this paper show that the antibodies to the tetanus poison can be produced in quantity by other cells than those of the central nervous system, for which, apparently, tetanus toxin has an especial affinity.