

PEKING BRANCH

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Studies in immunity to tetanus bacilli.

By CARL TENBROECK and JOHANNES H. BAUER.

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

So much emphasis has been placed on the part played by antitoxin in immunity that when we found this antibody in the sera of guinea pigs carrying tetanus bacilli in their intestinal tract, we assumed that those animals would be immune to all types of this organism. We felt all the more secure in this assumption since Tulloch¹ has shown that the toxin produced by one type of tetanus is the same as that produced by any of the other types in that they are all neutralized by one antitoxin. When put to a test we found that this assumption is not entirely correct, as is shown by the results outlined below.

We were not satisfied with the methods used by others for the production of tetanus as the incubation period was too short and the animals usually died within two or three days after inoculation. After considerable search we have adopted aleuronot as an irritating agent, when injected with a small number of spores, as symptoms appear on the fifth day, and death follows on the seventh or eighth day after inoculation.

All control animals as well as those used for testing the protective power of sera were bled, and their serum was shown to be free from antitoxin. In addition cultures of their feces were negative for tetanus-like bacilli.

As has already been pointed out² guinea pigs that carry one type of tetanus bacilli for about five months show appreciable amounts of antitoxin in their sera. When tested it is found that these animals are immune only to the type of bacilli which they

¹ Tulloch, W. J., *J. Hygiene*, 1920, xviii, 103.

² Ten Broeck, C., and Bauer, J. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxi, 267.

are carrying. When injected with any other types they die in the same length of time as the controls. On the other hand, guinea pigs fed all types of spores are immune to the three types with which we have been working, and it is probable that they are immune to the other three types.

We have produced type serum in rabbits, one set of animals being given repeated injections of washed spores, and the other repeated injections of washed bacilli from young, actively growing, cultures. We refer to these sera as anti-spore and anti-bacterial respectively. Some sera contained antitoxin, while others did not; and we were fortunate in securing for each type of bacillus an anti-spore and an anti-bacterial serum practically free from antitoxin. The anti-spore sera were free from agglutinins, while the anti-bacterial sera contained agglutinins for the type of bacillus injected. One-tenth cubic centimeter of either the anti-spore or the anti-bacterial serum injected intraperitoneally one hour before the introduction of tetanus spores into the muscle, will protect against the type spore used in the immunization, while three cubic centimeters will not, as a rule, protect against the other types. When cross protection occurs it is the anti-bacterial serum which has this property. This cross protection is not very marked as animals injected with three cubic centimeters of serum usually show tetanus, but recover.

When injected before the introduction of spores, the anti-spore serum has a slightly higher protective value than the anti-bacterial; but when injected after the spores, the anti-bacterial has the higher titer. One cubic centimeter of the latter injected three days after the spores are introduced will prevent development of tetanus if the spores are of the same type as those used in the preparation of the serum. In one instance two cubic centimeters injected into each of two guinea pigs four days after the introduction of spores prevented infection, whereas control animals had spasms on the fifth day, and were dead on the seventh. One cubic centimeter of anti-spore serum injected two days after the spores will protect but if injected on the third day there is slight protection, if any.

Guinea pigs that have been injected with a protecting serum followed by spores and aleuronot, and that have shown no tetanus or a tetanus from which they have recovered, are immune to the type used in the first inoculation, but not the other types. Ani-

mals that have recovered from a severe generalized tetanus show practically no antitoxin in their serums, but are immune to the type of organism that produced the primary infection. They are not immune to the other types.

Our results show that antitoxin plays little part in immunity to tetanus, but that there is another immune body or bodies *specific for type* which prevents infection. As yet we have not attempted to determine the nature of these anti-bodies. Tulloch, working with type sera produced by the injection of whole cultures, has shown that they markedly stimulate phagocytosis, and that this property is specific for type. It seems likely that our sera also act by stimulating phagocytosis. It is suggested that serum for the prevention and treatment of tetanus would be of greater value if the animals used for its production were injected with different types of tetanus bacilli in addition to the toxin.

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The hatching phenomena of *Clonorchis* ova.

By O. K. KHAW. (Introduced by E. C. Faust).

[*From the Parasitology Laboratory, Peking Union Medical College and from Amoy University, China.*]

Ova of *Clonorchis* have a high specific gravity, as they sink in concentrated saline solution within one-half hour, and in pure glycerine (Sp. gr. 1.26) in 48 hours. The shells show markings of an arabesque pattern, best brought out by staining with weak potassium permanganate of toluidine blue. They contain fully developed embryos at the time of discharge from the worm. In spite of these facts, under experimental conditions which may also hold true in nature, they would not hatch out but would degenerate if left undisturbed. Various external factors may operate upon these eggs either singly or in conjunction. The more important of these are as follows:

(a) Mechanical, *e. g.*, pressure of needles in teasing out the eggs; the pressure of cover-glass, or the running in of H₂O under the latter and subsequent streaming with filter-paper. A heavy