# EXALTATION OF AN ATTENUATED STRAIN OF TRYPANOSOMA BRUCEI.

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We think of a micro-organism as a more or less fixed definite thing undergoing changes which result in a difference of functions. Its characteristic properties may be enhanced or diminished; that is there may be an upward or a downward trend as to its activities. Let us, for instance, consider the yeast plant. Under varying conditions of temperature and media, it will produce different per cents of alcohol. The same principle is true for the properties causing the souring of milk, heat, light, gas, color production, etc.

Among the rest of the functions, is the change of an organism's ability to grow in the animal body. This is termed virulence, and when it is intensified it is referred to as exaltation, and when the virulence is lessened, decreased, it is known as attenuation. The latter is brought about relatively easily and the methods of so doing are rather numerous, while it is much more difficult and the ways are fewer to exalt an organism.

**Exaltation:** The enhancing of the virulence of micro-organisms may be accomplished by repeated serial passage through susceptible animals. An excellent example of this is seen in Pasteur's work of standardizing the varying so-called "street virus" of Rabies, Hydrophobia, by repeated transfer to rabbits until it killed these animals uniformly on the sixth or seventh day. It required 270 such passages in order to definitely establish its virulence. Each species of animal is in a class by itself in this respect. The bacillus of hog erysipelas was standardized in pigeons.

In some instances it is difficult to bring about exaltation in this way because the parasite is present only in very small numbers and a reasonable dose of the germ may not be able to break down the animal's normal resistance.

Animal passage along with another organism (microbic association) at times accomplishes the desired result where other methods fail. That usually is the case in tetanus and is important in intestinal infections. In the latter case, the vibiro of Asiatic cholera when associated with another organism results in a perfect infection, whereas the disease otherwise is produced with difficulty.

Another method which to some extent is similar to the one just mentioned is the introduction of the organism into the animal body plus a chemical such as lactic acid. A lactic acid producing organism might give rise to the same desired reaction.

Passage *in vivo* with the organism enclosed within collodion or other semi-permeable membranes as expounded by the author in a previous

"Proc. Ind. Acad. Sci., vol. 34, 1924 (1925)."

article[1] also gives excellent results. Similarly the inoculation of the organism into an area in the body, the sole of the foot, where the phagocytes do not enter, may increase its disease-producing qualities. Frequent consecutive transplantation of the organism upon a medium containing blood serum and other body fluids tends to increase its pathogenic properties, especially if cultivation has been upon ordinary laboratory media.

**Attenuation:** The weakening of an organism as regards to certain properties was first quite accidentally discovered. Pasteur '80 and his associates during the summer set aside their culture of chicken cholera and when their work with it was resumed in the fall, found that it was harmless when injected in fowl.

This effect upon other organisms was soon studied and now, of course, it is a common occurrence.

Growth upon artificial media is unfavorable to and always results in attenuation of the pathogenic organisms. In some cases a very few passages *in vitro* will suffice to destroy pathogenicity. Such organisms as cholera, typhoid, and many others soon lose their infectivity powers. Anthrax loses its toxicity slowly, as does the bacillus of tuberculosis. *Trypanosoma Brucei*, to which the latter part of this article will be devoted, required three years before a perceptible change in pathogenicity was noticed.

If instead of selecting a very susceptible, a relatively insusceptible (resistant) animal is used, attenuation of the organism ensues. The classical example of this is the smallpox virus attenuation by passage through the heifer.

Temperature plays an important role. We know that 60° C. for a short time will kill pathogenic organisms. And consequently, if we heat at 55° C. or 50° or even at 45° for a certain length of time, attenuation will occur. Most pathogenic organisms prefer to grow at body temperature (37½° C.), so that if we incubate them at 40-42° C. deteriorating results follow.

Heat may not be the only deciding factor. Oxygen or its absence, and especially metabolic products, have their effect.

Growth in gradually increasing concentrations of chemical substances such as phenol will cause organisms to lessen their activities.

Most of the organisms, if not all, are seriously affected if not killed by exposure to direct sunlight; even ordinary light has its effects.

Dessication almost instantly kills the vibiro of cholera and affects other organisms.

Pasteur utilized this principle in his rabies treatment. He dried the spinal cord containing the virulent virus over caustic soda and obtained varying degrees of attenuation.

An interesting and important point in connection with exaltation and attenuation is in the case of *Trypanosoma Brucei*, the cause of nagana in cattle.[2]

[1] 37th Proc. Ind. Acad. Sci., pp. 69-73, 1921.

[2] 38th Proc. Ind. Acad. Sci., pp. 233-245, 1922.

This one-celled flagellate after three and one-half years' cultivation *in vitro*[3] became markedly attenuated so that it no longer infected rabbits, and was practically avirulent for the guinea pig. It, however, was slightly pathogenic for the extremely susceptible rat.

A pair of rats were inoculated with this markedly attenuated strain of nagana and two sets of experiments, A and B, were carried out.

In set A, rats were injected with the blood taken from the infected animal at the first appearance of the parasite in the blood stream, which was ten days after the inoculation.

As soon as the trypanosome was observed in the blood of the second pair of rats, two more animals were inoculated. In like manner other pairs of healthy rats were injected. That is, in all cases, the rats were inoculated with blood taken from infected rats as soon as the presence of the trypanosome could be demonstrated and at no time was the number of parasites great (1/10 or 1/20 per field, number 7 objective).

In a similar way in set B, healthy rats were injected with trypanosomal blood just before the infection proved fatal when the organisms were extremely numerous (about 200 per field).

In set A, it required 34 serial animal passages to restore the trypanosome to its original pathogenicity so that it killed rats regularly from three to five days. While in set B, its virulence was fully regained after eight such passages.

The outcome of these experiments can be explained from several standpoints. First, in set A, the parasite was present in very small numbers and a reasonable dose was not able to break down the animal's normal resistance, while in set B, the number of organisms was always large and consequently the rat's refractoriness was easily overcome. Second, in trypanosomiasis there is practically no demonstrable degree of immunity which in no way might interfere with the development of the parasite. Thirdly, the virulence of the trypanosome in the experiments carried out under B was being constantly enhanced by its development in the same rat, that is, it was the equivalent to serial passage of the parasite through a susceptible animal body. This could not occur in experiments conducted in set A.

[3] Jour. of Infect. Diseases, Vol. 15, No. 1, July, 1914, pp. 24-62.