German Medical Weekly

Thursday, #49, 4th of December, 1890

From the hygienic institute of Herrn Geheimerath Koch in Berlin:
Concerning Development of Diphtheria Immunity and Tetanus Immunity in Animals

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During our lengthy and ongoing studies of diphtheria (Behring) and tetanus (Kitasato), we have gained knowledge about questions of therapy and immunity. In both of these infectious diseases, we have been able to cure infected animals as well as to pretreat healthy animals in such a way that they do not become ill with diphtheria or tetanus.

In which fashion the cure and the immunization can be attained, we will only describe here\(^1\) [superscript: more detailed information about this will follow in the Zeitschrift für Hygiene] in as much detail as is necessary to prove the correctness of the following sentence.

“The immunity of rabbits and mice that have been immunized against tetanus is based on the ability of cell-free blood fluid to render harmless the toxic substances which the tetanus bacilli produce”

The explanation for the immunity which is described in the previous sentence was not yet considered in recent work which concerned itself with the immunity question.

In addition to the the study of phagocytosis (which looked for an explantation in the vital activity of cells) the other explanations [for immunity] were the antibacterial effects of blood and the ability of the animal organism to become accustomed to poisons.

When one of the above explanatory principals was not sufficient, or was shown to be incorrect by experimentally-working-authors, it was felt reasonable, by means of exclusion, to take into account the other explanation. For example, Bouchard, in his speech at the Tenth International Medical Congress, which reflects most concisely perhaps the current status of the immunity question, said: [In French] “Thus, let us not talk anymore of the attraction of leucocytes and the habituation of nervous cells to bacterial poison: this is pure rhetoric” and :”it is in effect this bactericidal state which constitutes vaccination or acquired immunity”

This positive explanation is the equivalent of what Roger\(^2\) [footnote 2: Contribution a l’étude de l’immunité acquise] expressed earlier with the following words: [French] “Vaccination produces in the organism chemical modifications which rend the humours and the tissues unfavorable to the vegetation of the microbe against which one has protected the animal.”
Now one of us (Behring) noted through his studies of diphtheria-immune rats and immunized guinea pigs, that none of the above mentioned theories was capable of explaining the immunity of these animals and he found himself forced to search for a different expulatory principle. After multiple failed efforts, the diphtheria-poison-destroying effect of the blood of diphtheria-immune animals showed in which direction the resistance to diphtheria was to be sought. But only after the application to tetanus of the experiences made with diphtheria did we arrive at results that, as far as we can tell, are conclusive.

The experiments described in the following prove:

1. Blood from tetanus-immune rabbits assesses tetanus-toxin-destroying properties.
2. These properties can also be demonstrated in extra-vascular blood and in the cell-free serum that can be obtained from it.
3. These properties are of such persistent nature that they remain effective in the organisms of other animals, so that one can, through blood or serum transfusion, obtain outstanding therapeutic effects.
4. The tetanus-poison-destroying properties are lacking in those animals that are not immune to tetanus and when one administers tetanus toxin to non-immunized animals, it can be found even after death of these animals in their blood and other body fluids.

As proof for these statements we report the following large series of experiments:

In a fashion that is exactly described elsewhere (footnote 1-in a article to appear soon in the publication of hygiene by Kitasato), a rabbit was immunized against tetanus. As proof of the degree of immunity this rabbit received 10cc of a virulent infectious tetanus bacillus culture of which 0.5 cc would suffice to definitely kill a normal rabbit. This rabbit remained entirely healthy.

This did not only achieve immunity against infection with living tetanus bacilli but also against tetanus toxin; because it tolerated 20 times the amount of this toxin that would have sufficed to kill normal rabbits without exception (subscript...)

Blood was taken from the carotid artery of this rabbit.

One mouse received 0.2cc, another received 0.5cc, in the peritoneal cavity of this fluid blood before coagulation. Both of these mice, along with two control mice were injected with virulent tetanus bacilli 24 hours later of such strength the control mice became ill after 20 hours and died after 36 hours. Both pre-treated mice on the other hand remained healthy.

The larger quantity of this blood was allowed to stand until serum had separated.
Six mice received 0.2cc of this serum injected into the peritoneal cavity; after the administered infection 24 hours later all six remained healthy, whereas control mice all died of tetanus within 48 hours.

With this serum furthermore, therapeutic success can be achieved when the animals are first infected and then receive the serum injected into the peritoneal cavity.

Furthermore, we have carried out experiments that show the enormous toxin-destroying-effect of this serum.

A ten day tetanus culture, which was filtered to become germ-free, produced liquid of which 0.00005cc would kill a mouse after 4 days and 0.0001cc in less than two days.

Now we mixed 5cc of serum from tetanus immune rabbits with 1 cc of this culture and allowed the serum to work on the tetanus toxin contained in the culture for 24 hours. Of this mixture four mice received 0.2 cc each containing 0.033 of culture or more than 300 times the fatal dose for mice; all 4 mice remained healthy. The control mice, however all died from 0.0001 cc of the culture within 36 hours.

The mice from all of the above mentioned experiments, those that received the serum in the peritoneal cavity, as well as those that received the mixture of tetanus toxin with serum and remained persistently immune as far as we can tell at present; they survived later repeated injections with virulent tetanus bacilli without showing even a trace of the illness.

This fact is particularly noteworthy because in the numerous individual experiments no mouse, no rabbit, and in general no animal tested up until now was found to be immune to tetanus and also because the multiple attempts in this institute to immunize various animals against tetanus were completely without success.

We can therefore conclude that the above expressed description of the mechanism of immunity which led to the discovery of immunization method that was effective and harmless, is sufficient to satisfy a very rigorous proof of causality.

Of course at all points control experiments were carried out with the blood and the serum of non immune rabbits. This blood and serum proved to be both therapeutically and in its effects on tetanus toxin, completely ineffective.

The same is true for bovine, calf, horse, and hammel (?) serum as particular experiments have shown.

Also the intravascular blood of living, non-immune animals possesses no tetanus-toxin-destroying properties as shown by the following multiply-repeated experiment: Rabbits which received 0.5cc of a toxin containing germ free tetanus culture injected subcutaneously die after 5 to 6 days with typical tetanus features. At autopsy one finds almost without exception a serous transudate in the thoracic cavity. On the average 0.3cc
of this transudate are enough to sicken and kill a mouse with tetanus and in the same dose the blood of the tetanus-infected animal produces tetanus in mice.

We refrain at this point from drawing the consequences of our results which may be useful for the treatment of humans with diphtheria and tetanus, much as they have been shown to yield therapeutic-effective products in animals.

In conclusion we would like to make one more point.

In times gone by, blood transfusion was considered to be a heroic but in some cases effective treatment; more recently it is believed that one can achieve the same thing with physiological saline solutions. The results of our experiments warn us clearly to remember the words “blood is a very special juice”.