THE EFFECTS OF INJECTION OF ANTITOXIC AND ANTIBACTERIAL SERA ON THE OPSONIC POWER OF THE BLOOD

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Last January, owing to an accidental inoculation with a culture of tetanus bacilli, one of us had a prophylactic dose of 10 c.c. of antitetanic serum. As we were working at the opsonic index at the time, it occurred to us that it would be interesting to note the effect, if any, of this injection on the opsonic power of the blood to tetanus bacilli.

Two days elapsed after the injection before the first observation was made, but from this time onwards a daily examination of the blood was undertaken. On the first day, as is shown in the accompanying chart, the tetano-opsonic index was considerably raised. On each succeeding day it fell, and on the sixth day after injection it was found to be below normal ($\cdot 7$). On the eighth day the individual who had the injection of antitetanic serum, whilst using his own blood as a control, discovered that his tuberculo-opsonic power was considerably depressed. An estimation was immediately made, not only with tubercle but also with staphylococci, and his index was found to be low to both organisms. In passing it might be mentioned that before injection his index to tubercle was normal.

To continue—on the tenth day his tetano- and staphylo-opsonic indices were still lower, and it is interesting to note that coincidentally with the low opsonic power of the blood, a marked urticarial, and on the following day a purpuric rash broke out all over the body. The examination of the opsonic power of the blood during the following days showed a gradual return to normal. (See Fig. 1).

Having noticed these facts in connection with the injection of antitetanic serum, we then proceeded to see if similar changes would occur with the use of antistreptococcic serum. The streptococcus used for this purpose was obtained from the blood of a patient suffering from malignant endocarditis. After finding that the opsonic

Fig 1.—Chart showing the effect of the injection of 10 c.c. of antitoxic serum on the opsonic index.

index of a man was normal to this streptococcus on three consecutive days, 10 c.c. of antistreptococcic serum was administered subcutaneously. No effect on the opsonic index was noticed until the seventh day, when it had fallen to .5.

The index to *B. coli* which had previously been shown to be normal was now found to be low also. This depression of the index continued until the eleventh day, when on examining the films we found that the washed leucocytes besides containing bacteria had also ingested red blood corpuscles. This phagocytosis of the red blood corpuscles was observed for several weeks. Two or three days later, we incubated for fifteen minutes, equal quantities of washed leucocytes obtained from several individuals, among them from the patient himself, with equal volumes of his (the patient's) serum. In some of these the red corpuscles were noticed inside the leucocytes, in others they were not. There were no red corpuscles observed in the leucocytes obtained from the patient himself.

As in the case of the antitetanic serum certain general effects were noticed, namely :—The patient injected, who was suffering at the time from interstitial nephritis, had a decided increase in the amount of albumin in his urine and developed a little oedema about the same time as the phagocytosis of the red corpuscles was observed. Ordinary blood films made from the patient at the same time shewed nothing abnormal.

As a rise in the tetano-opsonic index occurred after injection of antitetanic serum it may seem strange that there should be no similar rise with antistreptococcic serum, but since there are probably several varieties of streptococci it was thought that possibly the particular strain of streptococcus which was used had not been employed in the preparation of the antistreptococcic serum. In connection with this it might be mentioned that the patient from whose blood the streptococcus used in this experiment was obtained had been treated with the antistreptococcic serum for several days without effect. The experiment therefore was repeated in an exactly similar manner with the exception that the streptococcus used was procured from a case of erysipelas. In this instance there was a definite rise in the index on the fifth day after injection from normal to 1·7 as shewn in the chart. (Fig. 2).

The later effects were very similar to the former, the red blood corpuscles being noted inside the leucocytes about the eleventh day,

though in this case it was not nearly so marked as in the preceding. On referring to the chart it will be noticed that the fall of the opsonic power both to the streptococci and *B. coli* was not so great as in the former instance. This, together with the less marked ingestion of the red blood corpuscles, might possibly be accounted for by the fact that in the latter experiment the person's kidneys were normal, and so the excretion of toxins from the blood would be more readily accomplished.

Fig. 2.—Chart showing the effect of the administration of 10 c.c. of anti-streptococcal serum on the opsonic index.

We next attempted to ascertain the effect of antidiphtheritic serum on the opsonic index. After finding that a certain person was normal to the diphtheria bacillus on three consecutive days, 2,000 units of

antidiphtheritic serum was administered. The first result was a fall in the diphthero-opsonic index to $\cdot 7$.

This was followed on the fourth day after injection by a rise which reached 1.6, and finally there was a secondary fall due to the general poisonous effects of the serum appearing about the tenth day.

As in the case of the antitetanic and antistreptococcic serum the opsonic index to other organisms was also lowered.

The serum of the patient mixed with washed corpuscles also produced in a slight degree a phagocytosis of the red blood corpuscles.

Another person was injected and the experiment repeated in an exactly similar manner and substantially the same results were obtained, the only difference being that the rise in the opsonic index was not so marked.

The conclusions we draw from these observations are :—

1. That the rise in the opsonic index following the injection of the antitoxic and antibacterial sera, is, on the whole, comparatively slight.

2. That the depressing effects are more marked.

These effects cannot altogether depend upon the injection into the human body of a foreign serum, as they were much more marked in the case of antitetanic serum than in the other two. The probability is that the injection of the tetanus toxin into a horse, causes it to form some substance which is very toxic to the human body. These toxic effects are indicated by :—

- (a) Several symptoms, *e.g.*, skin rashes, albuminuria, oedema, and a feeling of great debility.
- (b) Lowering of the opsonic index to most of the common organisms.
- (c) By something in the serum which produces an injurious effect on the red blood corpuscles and thus enables the leucocytes to attack and absorb them.

At first sight it is not easy to see why, after the injection of the various sera, there should be a rise in the opsonic index at all. This may perhaps be explained in one of two ways. Firstly, that the various antitoxic antibacterial sera contain opsonins.

In order to find out whether such is the case or not, these sera (antistreptococcic and antidiphtheric) were taken instead of human serum and were incubated for 15 minutes with equal parts of washed leucocytes and an emulsion of the particular organism, *i.e.*, diphtheria and streptococcus. No phagocytosis was observed. This seems to indicate one of two things :—

1. That the various sera, although they must have contained opsonins when freshly prepared (as they are made by injecting horses with what is practically a vaccine) may not after having been kept for several months contain any ; or

2. That human leucocytes will not exert their phagocytic power in the presence of a serum other than human.

However, it was found that washed human leucocytes will take up bacteria in the presence of freshly drawn cat's serum. Using human leucocytes and an emulsion of staphylococci it was ascertained that the opsonic index of cat's serum as compared with human was $\cdot 6$ thus shewing that human corpuscles have a considerable phagocytic action in the presence of cat's serum.

This would seem to show that the various antitoxic and antibacterial sera which, as is usually the case, have been kept for at least several weeks before use, do not contain any opsonins. Therefore the rise in the opsonic index which was noted may be due to either :—

- (a) Substance in the sera which is easily converted by the body into opsonins, or
- (b) As is more likely, to vaccines which are present in the antitoxic and antibacterial sera and which when injected into the body stimulate it to form opsonins.