EXPERIMENTAL EVIDENCE OF THE LOCAL PRODUCTION OF 'OPSONINS'

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These experiments were originally undertaken in the hope that we might, by the firm application of an elastic bandage and the subsequent inoculation of a vaccine, be able to demonstrate the local production of specific 'opsonins.' Before proceeding to inoculate we felt that it was necessary to test the local and general effects upon the opsonic index produced by the bandage alone. The alterations in the index were so marked that our original purpose was frustrated, as it would have been impossible to say to what extent variations obtained were due to the vaccine.

We are, therefore, left with a series of experiments demonstrating the local and general effects upon the opsonic index resulting from the application to a normal individual of a Bier's bandage. The experiments may be divided into three groups.

A. Evidence that the index of the subject was normal to the *Diplococcus Intracellularis Meningitidis* (Weichselbaum).¹

B. Local and general effects upon the index when an elastic bandage was applied with extreme severity.

C. Local and general effects upon the index when an elastic bandage was applied with less severity.

In all the experiments the emulsion was made from a twenty-four hours' old 'nasgar'² culture. Leucocytes (2 vols.), emulsion (1 vol.),

1. We selected the *Meningococcus* because we had already worked with it continuously for nine months in the treatment of patients, and were also so engaged at the time of commencing the experiments.

2 A. Ascitic fluid, 15 c.c.; distilled water, 35 c.c.; nutrose, 1 gramme. B. Ordinary peptone agar. Mix one part of A with two parts of B, steam thirty minutes, filter, place in tubes. Sterilise. (Gordon)

and serum (1 vol.) were always freshly prepared (not more than four hours old), and were incubated for ten minutes at 37° C. One hundred leucocytes were counted. In the majority of the experiments the counter did not know until he had finished to which persons the various films referred, as the slides were mixed with those of patients under treatment. As a rule, he did not know his figures at all, as they were taken down for him by another person. The slides were stained by the following method:—Twenty drops of Leishman's stain for thirty-five seconds; ten drops of distilled water added, carefully mixed and left for six minutes; washed in three changes of distilled water for twenty seconds altogether, and dried high up over a bunsen flame.

A.—PROOF THAT THE INDEX OF THE SUBJECT WAS NORMAL TO THE
MENINGOCOCCUS

Six preliminary observations were made, four on successive days. (See No. 1 on chart.) The culture employed on the first four occasions had been derived from a case seven days previously; that on the last two had been isolated 138 days before the first of these experiments. A different control blood was used on each occasion. Twice there was only a difference of one coccus between the subject and the control, viz., 135-136 and 229-228. The subject had, then, an index normal (0.9-1.1) to two strains of meningococcus of markedly different ages. The subsequent experiments confirmed this, as the index was always taken before the application of the bandage, and invariably fell within these limits.

B.—LOCAL AND GENERAL EFFECTS UPON THE INDEX WHEN AN ELASTIC
BANDAGE WAS APPLIED WITH EXTREME SEVERITY

(See Nos. 2, 3 and 4 on chart)

Five yards of Esmarch's plain rubber bandage were applied to the left forearm just below the elbow for an hour. Severe pain was induced in the hand during the experiment, and tingling and numbness for several hours afterwards. The subject was covered with cold perspiration, and was somewhat collapsed. The limb below

the bandage became intensely cyanosed at first, but towards the end of the hour was leaden-coloured. The pulse was absent at the wrist, and sufficient blood for the index was only obtained by pricking deeply the pulps of all the fingers.

Results.—In each case there was a very distinct rise in the index of the left hand at the end of the hour (1·7, 1·5, 1·9). In one case (Experiment No. 4) this rise had already begun at the end of half an hour. In this experiment indices were taken from both hands, and showed that the rise was confined to the left. Experiments Nos. 2 and 4 demonstrate that twenty-four hours later the index had returned to normal.

C.—LOCAL AND GENERAL EFFECTS UPON THE INDEX WHEN THE
BANDAGE WAS APPLIED WITH LESS SEVERITY

(See Nos. 5, 6, 7, 8, and 9 on chart)

In these experiments the bandage was applied with sufficient firmness to produce apparently the same amount of stasis and discoloration, but relatively little pain. That the constriction was sufficient to isolate the limb from the general circulation is seen in the chart, which shows that variations in the index, where they occurred at all, were confined to the left hand.

Experiments Nos. 5 and 9 show no result. In No. 5 the index readings are worth reporting in detail, as showing the consistency of the results. Control: 77 cocci in 100 leucocytes. Four readings from the subject (both hands) at the various stages of the experiment: 79, 76, 74, and 78 cocci in 100 leucocytes. In No. 9 a rise to 1·2 on the second day can hardly have been due to the application of the bandage.

If we consider Experiments Nos. 5, 6, 7, 8, and 9 together, we find that under the conditions of the observations the rise in the index is never more than moderate, and may be absent. In none of the experiments does the effect last more than twenty-four hours.

CONCLUSIONS

It would appear that to produce a marked rise in the index a Bier's bandage when applied for one hour must be sufficiently firm to cause very considerable pain. This leaves untouched the question of a more moderate application extending over a longer period of time ; but, from a consideration of the above experiments and the variations in the rises obtained, it may be doubted whether an application however long which does not isolate the limb will have any appreciable effect on the index. It is certain that the results will be irregular in character. The irregularity of the indices seems to us to be of interest, if only from the fact that it confirms the clinical experience of those who use Bier's bandage as a therapeutic measure, and find that the beneficial effects vary greatly.

A further point is brought out, namely, that the alteration in amount of the ' opsonins ' (or such part of them as can be gauged by the index) was purely local, and that, therefore, no other tissues of the body were required for its manifestation than those present in the bandaged forearm.

OPSONIC INDEX CHART

S Index before experiment.
 --- Index curve of left hand.
 — Index curve of right hand.

X Index of left hand half an hour after application of bandage.
 O Index of either hand at time of removal of bandage.
 ● Index of either hand one hour after removal of bandage and on succeeding days.