

A quantum of sterilised staphylococcus culture corresponding to 2,500,000,000 of staphylococci was inoculated.

Three days afterwards his phagocytic index stood at 0·88.

A further quantum of 2,000,000,000 staphylococci was inoculated.

On the fifth and again on the eleventh day after inoculation the patient's phagocytic index stood respectively at 1·9 and 1·95.

Improvement in the patient's boils was already apparent at the date of the second inoculation. After this they completely aborted.

The patient afterwards relapsed, but did not come up for further observation.

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“On the Action exerted upon the Tubercle Bacillus by Human Blood Fluids, and on the Elaboration of Protective Elements in the Human Organism in Response to Inoculations of a Tubercle Vaccine.” By A. E. WRIGHT, M.D., late Professor of Pathology, Army Medical School, Netley, Pathologist to St. Mary's Hospital, Paddington, and STEWART R. DOUGLAS, M.R.C.S., Captain, Indian Medical Service. Communicated by Sir J. BURDON SANDERSON, Bart., F.R.S. Received July 26, 1904.

(From the Pathological Laboratory of St. Mary's Hospital, London, W.)

We propose to consider in this communication (1) the action exerted upon the tubercle bacillus by normal human blood fluids and the tuberculotropic\* substances which come here into consideration ; (2) the action exerted upon the tubercle bacillus by the blood fluids of those who are the subject of tubercular infection ; (3) the distribution of tuberculotropic substances in the infected organism ; (4) the question as to whether these protective substances are present in the blood of the infant at birth ; and (5) some points in connection with the elaboration in the human organism of tuberculotropic substances in response to inoculations of a tubercle vaccine.

I.—*On the Tuberculotropic Elements of Human Blood and on the Content of the Normal Blood in these Elements.*

*Agglutinins.*—The technical difficulties created by the circumstance that the tubercle bacillus grows in artificial culture in agglomerated masses stood for a long time in the way of the demonstration of the presence of agglutinins in the serum. These difficulties were for the

\* The term *tuberculo-tropic* is, in accordance with the scheme of terminology introduced by Ehrlich, employed by us to connote the property of *turning towards* and entering into chemical combination with the tubercle bacillus.

first time overcome by Arloing, who obtained, by the operation of a process of selection, a strain of tubercle which gives a homogeneous growth when the culture is frequently shaken up. For the homogeneous cultures of Arloing, Koch substituted a homogeneous suspension of bacillary fragments obtained by the trituration of ordinary tubercle cultures. Koch made his suspension with physiological salt solution.

The test fluid thus constituted exhibits—and this point did not escape the observation of Koch—a proneness to spontaneous agglutination. This defect, and it is a defect which may invalidate the results of any test examination, can, as was pointed out by one of us,\* be eliminated by employing in lieu of the 0·85-per-cent. NaCl solution, prescribed by Koch, a 0·1-per-cent. NaCl solution.†

We have in the case of the investigations on agglutination which are embodied in this paper in every case employed this 0·1-per-cent. salt solution both for the dilution of the serum and for the suspension of the tubercle powder.

In some of our more recent experiments we have substituted for the test fluid constituted as above a homogeneous suspension of tubercle bacilli obtained by heating an ordinary tubercle culture to 60° C. for an hour, filtering off the bacterial growth, breaking up this last in a mortar with a solution of 0·1-per-cent. NaCl in 0·5-per-cent. carbolic acid, and finally centrifugalising to remove any bacterial masses which have not been resolved into their elements.

In experiments conducted with either the one or the other of these test fluids agglutination effects are obtained with normal human serum. Conducting the experiments in throttled capillary tubes by the method described by one of us,‡ and taking cognisance of the effect by naked-eye inspection, a complete sedimentation is generally obtained in the 2- and 4-fold dilution and incomplete sedimentation in the 8-fold dilution. With some normal bloods complete sedimentation is obtained up to the 16-fold dilution.

*Bactericidal Elements.*—Our investigations into the question as to the presence of a bactericidal element in human serum are as yet incomplete.

*Opsonins.*—As already brought out by us in previous papers,§ the

\* Wright 'Lancet,' July 25, 1903.

† The principle which suggested the replacement of the stronger by the weaker salt solution finds application, as one of us (S. B. D.) has recently elicited, also in the case of plague cultures. The spontaneous agglutination which has up to the present been a source of difficulty in measuring the agglutination effect exerted by sera upon plague cultures can be completely avoided by employing a 0·1-per-cent. solution of salt in lieu of the broth or physiological salt solution ordinarily employed.

‡ Wright, *loc. cit.*

§ 'Roy. Soc. Proc.,' vols. 72 and 73.

phagocytic effect obtained when bacteria are introduced into the blood is dependent upon an action exerted by the blood fluids directly upon the micro-organisms.

We have investigated this question also in connection with the tubercle bacillus. In doing so a two-fold technical difficulty confronted us:—*first*, a difficulty associated with the circumstance that the tubercle bacillus is available in ordinary cultures only in the form of agglomerated bacterial masses, and *secondly*, a difficulty associated with the circumstance that unaltered tubercle bacilli when they have been obtained in homogeneous suspension are agglutinated by the action of both serum and physiological salt solution.

The first difficulty can be surmounted by breaking up the bacterial masses in a mortar in a 0·1-per-cent. NaCl solution, *i.e.*, in a salt solution diluted up to the point at which it will no longer bring together by its agglutinating action tubercle bacilli which have been mechanically separated.

The second difficulty can be surmounted by heating the tubercle culture to 100° C.

We subjoin here a series of experiments (conducted before the procedure last mentioned was thought out), with living tubercle bacilli suspended in a 0·1-per-cent. NaCl solution. It will be seen that the difference between the phagocytic effect obtained with the unheated and the heated serum respectively is sufficiently pronounced to throw altogether into the background the source of disturbance which is associated with the presence of an agglutinating element in the serum.

#### PRELIMINARY EXPERIMENTS.

*In this series of experiments a homogeneous suspension of living tubercle bacilli was made by rubbing up a small quantity of a tubercle growth (obtained from a glycerine potato culture) in an agate mortar in 1-in-1000 NaCl solution, and then centrifugalising to get rid of the bacterial masses which had not been resolved into their elements.*

##### Experiment 1.

###### A.

|   |         |
|---|---------|
| A. E. W.'s unheated serum.....                  | 2 vols. |
| S. R. D.'s washed corpuscles.....               | 2 „     |
| Suspension of living tubercle bacilli .....     | 1 vol.  |
| Phagocytic index (average of 67 P.W.B.C.), 5·4. |         |

###### B.

|  |         |
|--|---------|
| A. E. W.'s serum heated to 60° C. for 20 mins. ... | 2 vols. |
| S. R. D.'s washed corpuscles.....                  | 2 „     |
| Suspension of living tubercle bacilli .....        | 1 vol.  |
| Phagocytic index (average of 30 P.W.B.C.), 0·75.   |         |

## Experiment 2.

## A.

|   |         |
|---|---------|
| S. R. D.'s unheated serum.....              | 2 vols. |
| S. R. D.'s washed corpuscles.....           | 2 „     |
| Suspension of living tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 16 P.W.B.C.), 17·3.

## B.

|   |         |
|---|---------|
| S. R. D.'s serum heated to 60° C. for 20 mins. .... | 2 vols. |
| S. R. D.'s washed corpuscles.....                   | 2 „     |
| Suspension of living tubercle bacilli .....         | 1 vol.  |

Phagocytic index (average of 37 P.W.B.C.), 3·0.

## Experiment 3.

## A.

|   |         |
|---|---------|
| C. J.'s unheated serum.....                 | 2 vols. |
| S. R. D.'s washed corpuscles.....           | 2 „     |
| Suspension of living tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 19 P.W.B.C.), 14.

## B.

|  |         |
|--|---------|
| C. J.'s serum heated to 60° C. for 20 mins. .... | 2 vols. |
| S. R. D.'s washed corpuscles.....                | 2 „     |
| Suspension of living tubercle bacilli.....       | 1 vol.  |

Phagocytic index (average of 37 P.W.B.C.), 3·0.

With cultures which have been exposed to a temperature of 100° C. precisely similar results are obtained, while an advantage is gained in the respect that the count is no longer rendered difficult by the massing together of the bacilli.

All the experiments hereafter subjoined have been carried out with such a culture, *i.e.*, a culture heated to 100°, broken up in 1-in-1000 NaCl solution, and centrifuged until all unresolved clumps had been carried down.

Our next series of experiments was undertaken with a view to determining whether the increased phagocytic effect obtained with the unheated serum is due to an action exerted by the serum directly upon the tubercle bacilli.

## Experiment 1.

## A.

|  |         |
|--|---------|
| S. R. D.'s unheated serum.....             | 2 vols. |
| A. E. W.'s washed corpuscles.....          | 2 „     |
| Suspension of heated tubercle bacilli..... | 1 vol.  |

Phagocytic index (average of 20 P.W.B.C.), 6·9.

## B.

|  |         |
|--|---------|
| S. R. D.'s unheated serum.....             | 2 vols. |
| Suspension of heated tubercle bacilli..... | 1 vol.  |

The above were digested together for 15 mins. at 37° C. ; were then heated to 60° C. for 10 mins. ; and finally 3 vols. of the mixture were added to

|  |         |
|--|---------|
| A. E. W.'s washed corpuscles .....         | 2 vols. |
| Phagocytic index (average of 31 P.W.B.C.), | 3·5.    |

## C.

|  |         |
|--|---------|
| S. R. D.'s unheated serum.....             | 2 vols. |
| Suspension of heated tubercle bacilli..... | 1 vol.  |

The above were immediately, after mixture, heated to 60° C. for 10 mins., and were then added to

|  |         |
|--|---------|
| A. E. W.'s washed corpuscles .....         | 2 vols. |
| Phagocytic index (average of 50 P.W.B.C.), | 0·16.   |

## Experiment 2.

## A.

|  |         |
|--|---------|
| A. E. W.'s unheated serum.....             | 2 vols. |
| A. E. W.'s washed corpuscles.....          | 2 „     |
| Suspension of heated tubercle bacilli..... | 1 vol.  |
| Phagocytic index (average of 49 P.W.B.C.), | 5·2.    |

## B.

|  |         |
|--|---------|
| A. E. W.'s unheated serum.....             | 2 vols. |
| Suspension of heated tubercle bacilli..... | 2 „     |

The above were digested together for 15 mins. at 37° C. ; were then heated to 60° C. for 10 mins. ; and finally 3 vols. of the mixture were added to

|  |         |
|--|---------|
| A. E. W.'s washed corpuscles.....          | 2 vols. |
| Phagocytic index (average of 40 P.W.B.C.), | 2·6.    |

## C.

|  |         |
|--|---------|
| A. E. W.'s unheated serum.....             | 2 vols. |
| Suspension of heated tubercle bacilli..... | 1 vol.  |

The above were immediately after mixture heated to 60° C. for 10 mins. ; and were then added to

|  |         |
|--|---------|
| A. E. W.'s washed corpuscles.....          | 2 vols. |
| Phagocytic index (average of 50 P.W.B.C.), | 0·34.   |

## Experiment 3.

## A.

|  |         |
|--|---------|
| H. B. S.'s unheated serum.....             | 2 vols. |
| A. E. W.'s washed corpuscles .....         | 2 „     |
| Suspension of heated tubercle bacilli..... | 1 vol.  |

Phagocytic index (average of 20 P.W.B.C.) 4·8.

## B.

|   |         |
|---|---------|
| H. B. S.'s unheated serum.....              | 2 vols. |
| Suspension of heated tubercle bacilli ..... | 1 vol.  |

The above were digested together for 15 mins. at 37° C.; were then heated to 60° C. for 10 mins.; and finally 3 vols. of the mixture were added to

|                                   |         |
|-----------------------------------|---------|
| A. E. W.'s washed corpuscles..... | 2 vols. |
|-----------------------------------|---------|

Phagocytic index (average of 30 P.W.B.C.), 2·6.

## C.

|  |         |
|--|---------|
| H. B. S.'s unheated serum .....            | 2 vols. |
| Suspension of heated tubercle bacilli..... | 1 vol.  |

The above were immediately after mixture heated together for 10 mins. to 60° C.; and were then added to

|                                   |         |
|-----------------------------------|---------|
| A. E. W.'s washed corpuscles..... | 2 vols. |
|-----------------------------------|---------|

Phagocytic index (average of 20 P.W.B.C.), 0·4.

It will be manifest that these experiments testify to an opsonic action exerted by the serum directly upon the tubercle bacilli.

The smaller phagocytic effect recorded in each experiment in B as compared with A is at present without explanation.

The experiment next subjoined indicates that it is the potency of the serum rather than the potency of the white corpuscles which determines the amount of phagocytosis. In this experiment the corpuscles of the tubercular patient's blood, and the corpuscles of the normal blood respectively were employed in A' and A'' in combination with their native blood fluid. In B' and B'' a reciprocal exchange of blood fluids was made.

## A'.

|  |         |
|--|---------|
| Patient's washed corpuscles .....          | 2 vols. |
| Patient's serum .....                      | 2 „     |
| Suspension of heated tubercle bacilli..... | 1 „     |

Phagocytic index (average of 36 P.W.B.C.), 0·66.

## A''.

|  |         |
|--|---------|
| A. E. W.'s washed corpuscles.....          | 2 vols. |
| A. E. W.'s serum .....                     | 2 „     |
| Suspension of heated tubercle bacilli..... | 1 vol.  |

Phagocytic index (average of 32 P.W.B.C.), 3·1.

## B'.

|   |         |
|---|---------|
| Patient's washed corpuscles.....            | 2 vols. |
| A. E. W.'s serum .....                      | 2 „     |
| Suspension of living tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 31 P.W.B.C.), 2·1.

## B''.

|   |         |
|---|---------|
| A. E. W.'s washed corpuscles.....           | 2 vols. |
| Patient's serum.....                        | 2 „     |
| Suspension of living tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 30 P.W.B.C.), 1·3.

It will be seen that the phagocytic effect obtained with the patient's white corpuscles (in A') was (in B') increased more than three-fold in consequence of the replacement of their native serum by that of the control blood. The phagocytic effect obtained with the white corpuscles of the control blood (in A'') was (in B'') diminished in an almost corresponding degree (approximately two and a half times), by the replacement of their native serum by that of the patient.

These results are, it may be pointed out, in conformity with those recorded in our previous paper\* in connection with the phagocytosis of the staphylococcus pyogenes.

## II.—*Action exerted upon the Tubercle Bacillus by the Blood-fluids of those who are the subject of Tubercular Infection.*

The blood fluids of the subjects of a particular bacterial infection may be expected to differ with respect to their content in bacteriotropic substances from the blood fluids of normal persons. An increased content in these elements may be expected in the case where there has been active response on the part of the machinery of immunisation to the stimulus of infection; diminished content (*a*) where that machinery is becoming exhausted, and (*b*) where infection is dependent upon a native, or at any rate, antecedent deficiency in protective substances.

*Agglutinating effect.*—We have in no instance found the agglutinating power of the blood of tubercular patients higher than that of certain normal bloods. Sometimes we have found it notably decreased. In three cases we have found it altogether absent. On the average we find that it does not differ sensibly from that of the normal blood.

It would seem to follow—and this conclusion is in conformity with that arrived at by others—that no indication as to the presence or absence of tubercular infection can be drawn from the measurement of the agglutinating power, unless perhaps in the case where that reaction is found to be quite absent.

\* 'Roy. Soc. Proc.' vol. 73, pp. 129 and 130.

*Opsonic effect.*—The measurement of the opsonic power of the blood fluids discloses very definite differences. We have not in any of the sub-joined patients recorded at the outset an opsonic power equal to that of our own bloods. On the contrary there has been in each such case a definite defect of opsonic power. The results of our observations on this point are embodied in the table below.

Table I.—Showing the Opsonic Power of the Blood Fluids in a Series of Tubercular Patients.

*The procedure adopted was to mix together in each case the patient's serum with a suspension of heated tubercle bacilli and with washed corpuscles derived from a normal person. In each case the opsonic power of the normal serum employed as a control (derived in practically every case from A. E. W. or S. R. D.) was taken as unity.*

| Serial number of the observation. | Form of the tubercular infection.                   | Opsonic index. |
|-----------------------------------|---|----------------|
| Case 1 .....                      | Tubercular peritonitis.....                         | 0·67           |
| „ 2 .....                         | Laryngeal phthisis.....                             | 0·6            |
| „ 3 .....                         | Psoas abscess.....                                  | 0·4            |
| „ 4*.....                         | Tubercular sycosis .....                            | 0·4            |
| „ 5 .....                         | Nasal and pharyngeal lupus .....                    | 0·56           |
| „ 6 .....                         | Tubercular cystitis.....                            | 0·8            |
| „ 7 .....                         | „ „ .....   | 0·6            |
| „ 8 .....                         | Generalised lupus.....                              | 0·5            |
| „ 9 .....                         | Lupus of hand .....                                 | 0·9            |
| „ 10 .....                        | Tubercular prostatitis .....                        | 0·8            |
| „ 11 .....                        | „ glands .....                                      | 0·85           |
| „ 12 .....                        | „ abscess of thigh .....                            | 0·64           |
| „ 13†.....                        | Lichen scrophulosorum .....                         | 0·56           |
| „ 14 .....                        | Pulmonary phthisis .....                            | 0·75           |
| „ 15 .....                        | „ „ .....   | 0·69           |
| „ 16‡.....                        | Tubercular infection of ovary and peritonitis ..... | 0·65           |
| „ 17 .....                        | „ abscesses of arm and chest wall... ..             | 0·56           |

As indicated above, a diminished content in bacteriotropic substances such as we have here on record may be ascribed either to the

\* It is interesting to note in connection with this case that the definite diagnosis of tubercular infection, which was based upon the histological structure of a piece of excised tissue, was anticipated in view of the inductions obtained from the phagocytic test recorded above.

The alternative diagnosis of staphylococcus infection was excluded by the fact that the patient was found to possess a normal opsonic index with respect to the staphylococcus.

† The patient gave a typical local and general reaction when inoculated for diagnostic purposes with a test dose of Koch's old tuberculin.

‡ The diagnosis was based upon a histological examination of the extirpated ovary.



exhaustion of the protective elements under the influence of the bacterial invasion ; or, alternatively, to an antecedent deficiency in these elements.

The following considerations appear to us here to plead in favour of interpreting the low opsonic power of the tubercular patients here in question as the occasion and not the consequence of infection.

(a) Very low phagocytic indices have been obtained where constitutional symptoms were absent or insignificant. The cases denoted by the serial numbers 3, 4, 5, and 8 are instances in point.

(b) We have in practically every case found it possible to increase by an inoculation of a tubercle vaccine, the opsonic power of a patient's blood fluids.

### III.—*Distribution of Tuberculotropic Substances in the Infected Organism.*

We have made the subjoined observations on this question. It will be seen that they are in consonance with the observations (see pp. 151—153) we have made in connection with the distribution of the staphylococcic opsonins in the infected organism, and with the induction that the bacteriotoxic pressure is always reduced in the actual foci of infection.

#### Observation 1.

The patient was a child *æt.* 2 years, affected with tubercular necrosis of the sternum, and with a tubercular abscess in the thigh.

##### A.

|   |         |
|---|---------|
| Serum obtained from blood drawn from finger . . . | 1 vol.  |
| Physiological salt solution . . . . .             | 1 „     |
| S. R. D.'s washed corpuscles . . . . .            | 2 vols. |
| Suspension of heated tubercle bacilli . . . . .   | 1 vol.  |
| Phagocytic index (average of 20 P.W.B.C.), 3·9.   |         |

##### B.

|   |         |
|---|---------|
| Supernatant fluid of pus withdrawn from abscess.. | 1 vol.  |
| Physiological salt solution . . . . .             | 1 „     |
| S. R. D.'s washed corpuscles . . . . .            | 2 vols. |
| Suspension of heated tubercle bacilli . . . . .   | 1 vol.  |
| Phagocytic index (average of 20 P.W.B.C.), 1·1.   |         |

#### Observation 2.

The patient was a young man suffering from a psoas abscess due to tubercular infection.

##### A.

|   |         |
|---|---------|
| Serum obtained from blood withdrawn from finger | 1 vol.  |
| Physiological salt solution . . . . .           | 1 „     |
| A. E. W.'s washed corpuscles . . . . .          | 2 vols. |
| Suspension of heated tubercle bacilli . . . . . | 2 „     |
| Phagocytic index (average of 40 P.W.B.C.), 0·6. |         |

## B.

|   |         |
|---|---------|
| Supernatant fluid of pus derived from sinus ..... | 1 vol.  |
| Physiological salt solution.....                  | 1 „     |
| A. E. W.'s washed corpuscles .....                | 2 vols. |
| Suspension of heated tubercle bacilli .....       | 2 „     |

Phagocytic index (40 P.W.B.C. searched), 0.

With a view to ascertaining whether the tissue lymph might not normally be poorer in opsonic substances than the serum, the following experiment was made:—

## A.

|   |         |
|---|---------|
| Serum of blood withdrawn from A. E. W.'s finger.. | 2 vols. |
| A. E. W.'s washed corpuscles .....                | 2 „     |
| Suspension of heated tubercle bacilli.....        | 1 vol.  |

Phagocytic index (average of 50 P.W.B.C.), 0·92.

## B.

|   |         |
|---|---------|
| Fluid from blister raised by friction upon A. E. W.'s<br>finger ..... | 2 vols. |
| A. E. W.'s washed corpuscles .....                                    | 2 „     |
| Suspension of heated tubercle bacilli .....                           | 1 vol.  |

Phagocytic index (average of 50 P.W.B.C.), 0·86.

## Observation 3.

The patient was a young man operated upon for ascites dependent upon extensive tubercular infection of the peritoneum.

## A.

|   |        |
|---|--------|
| Serum obtained from blood withdrawn from finger | 1 vol. |
| B. H. S.'s washed corpuscles .....              | 1 „    |
| Suspension of heated tubercle bacilli.....      | 1 „    |

Phagocytic index (average of 21 P.W.B.C.), 25·4.

## B.

|  |        |
|--|--------|
| Fluid withdrawn from peritoneum .....      | 1 vol. |
| B. H. S.'s washed corpuscles .....         | 1 „    |
| Suspension of heated tubercle bacilli..... | 1 „    |

Phagocytic index (average of 34 P.W.B.C.), 4·6.

It is interesting to bring into relation with the data of this last observation (*a*) the fact that the opsonic index of this patient's serum stood to the opsonic index of the control serum employed (A. E. W.'s) as 1·5 : 1 ; (*b*) the fact that the prognosis, so far as the restriction of the infection to the existing focus of disease, is comparatively favourable in cases of tubercular peritonitis ; and (*c*) the fact that a retrogression of the infection often follows in these cases upon the evacuation of the peritoneal fluid.

The *first* and *second* of these facts suggest that a reaction of immunisation may be set up by the absorption of vaccinating elements from the infected peritoneum. The *third* fact, and the same applies (*vide* Case 1, p. 152, of the foregoing paper) also in connection with the evacuation of abscesses may, perhaps, find its explanation in the data given above. It would be reasonable to expect that the flow of new and active lymph, which would follow upon the evacuation of the stagnant and exhausted lymph, would operate in the direction of checking the growth of invading micro-organisms.

IV.—*Question as to whether the Protective Substances which come into consideration in connection with Tubercle are present in the Blood of the Infant at Birth.*

In view of the asserted superior susceptibility of infants to tubercular infection, it appeared to us to be of interest to measure the respective opsonic power of mother and infant. We employed for this purpose blood taken from the umbilical cord and blood taken from the mother's finger immediately after completion of labour. Our observations are subjoined.

Observation 1.

A.

|   |         |
|---|---------|
| Serum from mother No. 1 .....                   | 2 vols. |
| A. E. W.'s washed corpuscles ..                 | 2 „     |
| Suspension of heated tubercle bacilli .....     | 1 vol.  |
| Phagocytic index (average of 45 P.W.B.C.), 1·6. |         |

B.

|  |         |
|--|---------|
| Serum of infant No. 1 .....                      | 2 vols. |
| A. E. W.'s washed corpuscles ..                  | 2 „     |
| Suspension of heated tubercle bacilli .....      | 1 vol.  |
| Phagocytic index (average of 50 P.W.B.C.), 1·38. |         |

Observation 2.

A.

|   |         |
|---|---------|
| Serum of mother No. 2 .....                     | 2 vols. |
| A. E. W.'s washed corpuscles ..                 | 2 „     |
| Suspension of heated tubercle bacilli .....     | 1 vol.  |
| Phagocytic index (average of 50 P.W.B.C.), 0·4. |         |

B.

|   |         |
|---|---------|
| Serum of infant No. 2 .....                       | 2 vols. |
| A. E. W.'s washed corpuscles ..                   | 2 „     |
| Suspension of heated tubercle bacilli .....       | 1 vol.  |
| Phagocytic index (average of 100 P.W.B.C.), 0·37. |         |

## Observation 3.

## A.

|   |         |
|---|---------|
| Serum of mother No. 3 .....                 | 2 vols. |
| J. F.'s washed corpuscles .....             | 2 „     |
| Suspension of heated tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 40 P.W.B.C.), 3·55.

## B.

|   |         |
|---|---------|
| Serum of child No. 3 .....                  | 2 vols. |
| J. F.'s washed corpuscles .....             | 2 „     |
| Suspension of heated tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 40 P.W.B.C.), 1·85.

## Observation 4.

## A.

|   |         |
|---|---------|
| Serum of mother No. 4 .....                 | 2 vols. |
| A. E. W.'s washed corpuscles .....          | 2 „     |
| Suspension of heated tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 31 P.W.B.C.), 17·9.

## B.

|   |         |
|---|---------|
| Serum of infant No. 4 .....                 | 2 vols. |
| A. E. W.'s washed corpuscles .....          | 2 „     |
| Suspension of heated tubercle bacilli ..... | 1 vol.  |

Phagocytic index (average of 30 P.W.B.C.), 10.

## Observation 5.

## A.

Serum of mother No. 4 agglutinates a suspension of fragments of tubercle bacilli completely in dilutions of 1 in 2, 4, 8, and 16; incompletely in a dilution of 1 in 32.

## B.

Serum of infant No. 4 agglutinates the same suspension completely in dilutions of 1 in 2 and 1 in 4; incompletely dilutions of 1 in 8 and 1 in 16.

V.—*On some points in connection with the Elaboration by the Human Organism of Tuberculotropic Elements in response to Inoculations of a Tubercle Vaccine.*

We propose to set down here in briefest outline the more important of the facts which have emerged in the course of a study of the blood changes elicited by inoculations of a tubercle vaccine undertaken for therapeutic purposes.

*Nature of the Tubercle Vaccine employed.*—A tubercle vaccine may be defined, with respect to its derivation and its effect upon the organism, as any derivative of the protoplasm of the tubercle bacillus, which is

capable of inducing an elaboration of tuberculotropic substances in the organism.

We may include under this definition :—

(1) Such a vaccine as would be arrived at by (a) sterilising a tubercle culture at 60° C. ; (b) breaking up the culture in a mortar in 0·1-per-cent. salt solution ; (c) centrifugalising to remove any residual bacterial masses ; (d) re-sterilising at 60° C. ; and (e) standardising by enumeration, or by centrifugalisation in graduated tubes in a sufficiently concentrated salt solution.

(2) The preparation, which is sold, as a therapeutic agent, under the name of Koch's new tuberculin or T.R. tuberculin.

This preparation consists, as is well known, of a fine suspension of triturated tubercle bacilli. The trituration to which the tubercle culture is subjected is employed with the two-fold object of sterilising the vaccine by a process of comminution, and of obtaining the fine suspension which is desired.

It is doubtful whether the first of these ends can be efficiently secured by any process of trituration. The homogeneous suspension which is desired can, as was shown above, be obtained by means other than the comminution of the bacilli by machinery.

(3) The preparation, which is now sold, chiefly for diagnostic uses, under the name of Koch's old tuberculin.\*

This preparation consists, as is well known, of the inspissated filtrate of a tubercle culture which has been grown for a period of weeks upon glycerinated broth, and which has afterwards been sterilised at 100° C.

Pending the working out of a vaccine upon the lines indicated in (1), the T.R. tuberculin has been the vaccinating material employed.

In our earlier experiments this preparation was simply diluted with sterilised salt solution.

In our later experiments we have—after satisfying ourselves that the vaccinal properties of Koch's preparation are unaffected by the adoption of such precautions—in every case heated the T.R. tuberculin to 60° C. for 1 hour, and have made our dilutions with a sterilised salt solution which had received an addition of 0·25 per cent. lysol.

\* The proposition that the old tuberculin may appropriately be denoted a vaccine derives its justification, first, from the consideration that the prolonged cultivation and the prolonged digestion of the culture which is involved in the process of manufacture must be associated with autolysis, and secondly, from the observations made in connection with Case 13 of Table I and the last patient in Table III.

In the former case, the opsonic index of the patient's blood stood at 0·56 immediately before the inoculation of 1 milligramme old tuberculin. It stood at 0·55 18 hours afterwards in the height of the febrile reaction, and at 1·01 3 days later.

In the latter case, the opsonic index of the blood stood at 0·67 immediately before the inoculation of 1 milligramme of the old tuberculin. It stood next day at 0·4, and 8 days later at 0·76.

*Principle upon which the Patients were Selected and General Procedure  
Followed in Connection with the Inoculations.*

We have in our selection of cases been guided by the desire to deal at first only with the most aggravated and seemingly intractable cases of localised tubercular infection, and only with cases which would furnish unambiguous objective evidence of any progress or regress of the infection.

The general procedure followed was to begin in each case after the measurement of the content of the patient's blood in tuberculo-tropic substances, with very small doses (generally 1/500th milligramme) of the vaccinating material, and to reinoculate at intervals of 10 days, retesting the blood on each occasion, and in the case of each patient expressing the results in the form of a curve.

In our earlier experiments, before we had elaborated the procedure for measuring the opsonic power of the blood, we were necessarily restricted to a measurement of the agglutinating power.

*Data Furnished by the Measurement of the Agglutinating Power in the  
Case of Patients Undergoing Anti-Tubercle Inoculation.*

The method of investigation here in question—and we would note that it had before us been employed by Koch in connection with inoculations of his T.R. tuberculin—furnishes, it seems to us, indications which have a certain value.

An increase in the agglutinating power of the blood is generally, as in the cases tabulated below, obtained in the course of a successful immunisation.

It is, however, to be noted that the rise in the agglutination curve may occur long subsequent to the achievement of very marked clinical improvement, and further that such clinical improvement may be obtained quite apart from any sensible increase in the agglutinating power of the blood.

Table II.—Showing the Agglutinating Power of the Blood in the case of a Series of Patients, before Inoculation, and after a Series of Inoculations of Tubercle Vaccine.

| Patient's initials. | Highest dilution of serum in which complete agglutination was obtained, and date of observation (in brackets). | Highest dilution of serum in which complete agglutination was obtained after the inoculations particularised in the next column, and date of observation (in brackets). | Doses of T.R. tuberculin, and dates of inoculations (in brackets).  |
|---------------------|--|---|---|
| A. R.               | 3    (2.12.03)   | 32    (23.3.04)   | mgram.*<br>0·0025    (2.12.03)<br>0·01    (17.12.03)<br>0·01    (4.1.04)<br>0·01    (14.1.04)<br>0·0075    (21.1.04)<br>0·0075    (1.2.04)<br>0·015    (15.2.04)<br>0·02    (1.3.04)<br>0·04    (11.3.04) |
| J. A.               | 0    (6.4.03)  | 64    (21.4.03)   | 0·002    (6.4.03)<br>0·01    (8.4.03)<br>0·02    (15.4.03)  |
| N. T.               | 4    (6.1.04)  | 64    (23.3.04)   | 0·003    (20.1.04)<br>0·005    (2.2.04)<br>0·01    (23.2.04)<br>0·015    (2.3.04)<br>0·02    (12.3.04)  |
| N. W.               | 8    (10.12.03)  | 32    (20.3.04)   | 0·01    (17.12.03)<br>0·0075    (4.1.04)<br>0·0075    (19.1.04)<br>0·01    (28.1.04)<br>0·01    (10.2.04)<br>0·015    (19.2.04)<br>0·02    (3.3.04)<br>0·02    (14.3.04)                                  |
| E. J.               | 4    (27.1.04)   | 32    (16.3.04)   | 0·003    (27.12.03)<br>0·0066    (11.1.04)<br>0·01    (23.1.00)<br>0·015    (1.3.04)  |
| E. S.               | 2    (17.4.03)   | 64    (30.4.03)   | 0·005    (21.4.03)<br>0·01    (24.4.03)   |
| M. O.               | 0    (15.12.03)  | 24    (17.2.04)   | 0·004    (23.12.03)<br>0·008    (5.1.04)<br>0·016    (21.1.04)  |

\* The weights here in question refer in each case to the weight of tubercle powder stated to be contained in the quantum of T.R. tuberculin administered.

In addition to furnishing indications of successful advance in the direction of immunisation, the measurement of the agglutinating power of the patient's blood may afford also indications of regress in the direction of increased susceptibility resulting from an overtaking of the machinery of immunisation.

In exemplification of this we may quote three passages from the history of a patient (E. S. Tables II and III) with tubercular infection of the kidney and bladder, whose agglutination curve was followed for nearly 18 months.

The patient in question, who had in association with the inoculations set forth in Table II put on 5 lbs. in weight, received on April 30, the date on which the record in Table II closes, 0.025 milligramme on May 5, 0.05 milligramme, and on May 13, 0.2 milligramme of T.R. tuberculin.

In association with the first two of these inoculations, the agglutination curve sank away rapidly from 64 to 8, the patient losing at the same time  $3\frac{1}{2}$  lbs. in weight, and suffering from considerable constitutional disturbance.

In association with the third of these inoculations the agglutinins disappeared entirely from the blood.

On a later occasion in the beginning of November, 1903; when the general condition of the patient had very markedly improved and when her body weight had increased by 23 lbs., a similar negative phase effect accompanied by constitutional disturbance was obtained in association with the inoculation of three 1 milligramme doses of the T.R. tuberculin on November 2, 6, and 11 respectively. Here the complete agglutination, which was on the first of these dates obtained in a 32-fold dilution of the serum, was obtained after the inoculations only in an 8-fold dilution.

Again, in the beginning of December when another attempt was made to press the inoculations, the agglutination curve, which had risen again to 32 after the inoculations referred to in the preceding paragraph, declined in consequence of two 1 milligramme inoculations, first to 8 and then to 2, and the patient's symptoms were aggravated.

A similar decline of the agglutination curve has come under observation also in other cases in association with the premature increase of the dose of vaccine, and with the shortening of the interval between successive inoculations.

*Data furnished by the measurement of the Opsonic Power of the Blood  
in the case of Patients undergoing Anti-tubercle Inoculations.*

Much more valuable than the indications which can be gleaned from the measurement of the agglutinating power of the blood are the indications furnished by a measurement of the opsonic power of the patient's blood. While the measurement of the agglutinating power



of the blood may fail to furnish indications of an abnormally low resisting power on the part of the untreated patient; and while it may yield only tardy information of the alterations effected in the blood fluids by inoculation; and while it may sometimes altogether fail to distinguish between the patient's condition before and after successful immunisation; the measurement of the opsonic power satisfies it would seem all these desiderata.

We have already seen in Table I that it distinguishes\* between the tubercular subject and the person with normal resistance. And we shall see in the table below that it furnishes prompt and clear indication of the negative phase which supervenes upon inoculation, and again of the positive phase which succeeds the negative phase wherever the organism possesses the necessary power of response. Furthermore the measurement of the opsonic power of the blood distinguishes clearly between the untreated tubercular patient and the patient who has made progress in the direction of immunisation. This will appear clearly on comparing, in Table III below, the opsonic indices achieved after inoculation with those set forth in Table I.

In conclusion we may note, that while we are jointly responsible for the observations set forth in Sections 1, 3, and 4 of this paper, the work which is embodied in Sections 2 and 5 has been separately undertaken by one of us.

\* It will not, however, invariably do so.

Table III.—Shows the Changes induced in the Opsonic Power of the Blood Fluids by the Inoculation of Tubercle Vaccine, and furnishes Illustration of the Fact that the Cumulative Increase of the Protective Elements which is desired can be achieved only by the Proper Regulation and Interspacing of the Successive Doses of Vaccine.

| Initials.     | Brief history of case.  | Dates and particulars of inoculations undertaken since March, 1904.  | Opsonic index (opsonic power of the normal blood —A. E. W.'s— used as a control was taken as =1). |
|---------------|---|--|---|
| E. J. . . . . | Patient, a man of 30, developed tubercular glands on the left side of neck and a tubercular abscess on the point of the shoulder in the autumn of 1902. He was admitted to hospital and was operated upon for the first time in January, 1903. The wounds becoming invaded with tubercle, and refusing to heal, while the area of infection gradually extended, six further (scraping, extirpating, and skin-grafting) operations were undertaken during the course of the year. In December, 1903, when patient came up for treatment by inoculation, the whole area from the point of the shoulder to the ear had been converted into an ulcerated surface, there was a deep crater undermining the angle of the jaw and the ear, the left side of the face was distorted with swelling, and the axilla was occupied by a gland as large as a pigeon's egg. The patient was very anæmic and emaciated. Steady improvement has been made under the inoculation treatment, the swelling of the face has almost entirely dispersed, the cavity under the jaw has healed up from the bottom, the gland in the axilla can no longer be felt, the ulcerated wound surfaces have almost completely closed, and the patient has the constitutional aspect of a healthy man. | <div> <div>mgram.*</div> <div> 28.3.04<br/>18.4.04<br/>25.4.04<br/>10.5.04<br/>7.6.04<br/>27.6.04<br/>15.7.04<br/>19.7.04 </div> </div> <div> 0.04<br/>0.1<br/>0.1<br/>0.1<br/>0.1<br/>nil<br/>0.02<br/>nil </div> | 0.73<br><br><br><br>1.6<br>1.45<br>1.15   |

*Note.*—During the period 7.6.04—15.7.04 the inoculations of tubercle vaccine were suspended while the patient was being immunised against the staphylococcus pyogenes, which, by its presence on wound, appeared to be preventing the process of healing.

|  |   |   |   |
|--|---|---|---|
| E. S. . . . .  | <p>Patient came into hospital in December, 1902, with the characteristic symptoms of an infection of the urinary tract. Tubercle bacilli were constantly present in phenomenal numbers in the urine, and a tubercular ulcer of the bladder was detected. Patient had been losing flesh and suffered from night sweats and from constant pain and frequency. Treatment by inoculation was begun on 21.4.03 and has been continued up to date, inoculations and examinations of the blood and urine being undertaken on an average once in 10 days.</p> <p>Under the treatment the tubercle bacilli in the urine have gradually diminished, and since the beginning of May they have been completely absent. The patient has increased 36 lbs. in weight and is now to all intents and purposes well, except for an infection of the urinary tract by the bacillus coli and pneumococcus, which is being dealt with by the inoculation of the appropriate vaccines.</p> | <p>25.3.04 0.5<br/>8.4.04 0.5<br/>18.4.04 0.5<br/>27.4.04 nil<br/>9.5.04 0.5<br/>16.04 0.5<br/>15.6.04 nil<br/>6.7.04 nil</p>     | <p>0.93<br/>0.4<br/>0.42<br/>0.77<br/>0.4<br/>1.3<br/>2.2</p>   |
| <p><i>Note.</i>—The results of the blood examinations bring out that the inoculations were here conducted with excessive doses or perhaps at too short intervals. It will be seen that the opsonic power of the patient's blood declined in consequence of the inoculation undertaken on 8.4.04 from 0.93 on that date to 0.4 on 18.4.04. On the date last mentioned another 0.5 millegramme was inoculated—and it may be noted in this connection that here as elsewhere it was often necessary to inoculate before the result of the blood examination was known. On 27.4.04 recovery was still incomplete, the patient being obviously poorly and the inoculation was postponed. When next examined 12 days later the opsonic power of the blood was found to have risen to 0.77. A further dose of 0.5 millegramme was then inoculated, with the result that the opsonic power was reduced again to 0.4 on 1.6.04. On this day the patient again received a dose of 0.5 millegramme, the effect of this became manifest 14 days after in the rise of the opsonic power to 1.3 and 36 days after in the rise of opsonic power to 2.2.</p> | <p>Patient, a woman 31 years of age, developed a tubercular infection of the glands of the neck at the age of 14.</p> <p>When the abscesses which formed in association with these were opened the overlying skin became invaded, the infection spread to other glands, and tubercular disease developed in the little finger of the right hand. The two terminal joints of this finger were removed when the patient was 16.</p> <p>When the patient was 19 she was treated with Koch's old tuberculin, receiving three to four inoculations a day (150 inoculations in all). As a result of these inoculations the patches of lupus on hand, neck and face became inflamed, a piece of bone sloughed out of the arm and the patient lost weight and became seriously ill. She remained in hospital in all 13 weeks.</p>   | <p>18.4.04 0.04<br/>3.5.04 0.05<br/>16.5.04 0.05<br/>1.6.04 0.05<br/>10.6.04 nil<br/>19.6.04 0.04<br/>6.7.04 0.04<br/>22.7.04</p> | <p>0.16<br/>0.6<br/>—<br/>0.78<br/>—<br/>—<br/>1.85<br/>1.7</p> |

\* The weights here in question refer in each case to the weight of tubercle powder stated to be contained in the quantum of T.R. tuberculin administered.

Table III—*continue*.

| Initials.  | Brief history of case.   | Dates and particulars of inoculations undertaken since March, 1904. | Opsonic index (opsonic power of the normal blood—A. E. W.'s—used as a control was taken as = 1). |
|------------|--|---|--|
|            | <p>Four years later energetic local treatment was adopted and scraping operations were undertaken upon the glands in the neck. In 1900 the Finsen light treatment was adopted, and was persevered in for 18 months. This effected an improvement in the condition of the face and neck, but the disease continued to extend in the deeper structures, and in particular in the bones of the left arm. Finally it became necessary to amputate this limb.</p> <p>The disease now broke out in the stump, on the point of the shoulder and in the chest wall, while it persisted all over both sides of the face and neck. After Röntgen rays had been tried unavailingly, recourse was had to inoculations of tubercle vaccine, the treatment being begun on December 10, 1903. After 6 months treatment the discharge from the stump and chest wall has practically ceased, the patch on the point of the shoulder has healed up, the face appears to be in a better condition, and the patient's general health, which was previously very unsatisfactory, has improved in a remarkable manner. Her body weight has steadily gone up and has now reached 141½ lbs., as much as 5 lbs. having on one occasion been gained in the interval between two successive inoculations.</p> | mgm.  |  |
| A. R. .... | Patient, when referred for treatment by antitubercular inoculations in December, 1903, was found to be an emaciated, anemic, physically and mentally undeveloped child of 19 affected by lupus of the nose, throat, angle of jaw, and feet and hands. The last were a mass of ulceration, the bones of the hand being involved in many places.   | 22.3.04<br>7.4.04<br>18.4.04<br>23.4.04<br>5.5.04                   | 0.02<br>0.05<br>0.1<br>nil<br>0.05<br><br>1.1<br>0.25<br>—<br>0.61                               |

After 6 months' treatment the patches of lupus on the nose and throat have almost dried up and the condition of the hands and feet is much amended.

|         |      |      |
|---------|------|------|
| 6.5.04  | nil  | 0.85 |
| 16.5.04 | 0.1  | —    |
| 7.6.04  | 0.1  | 1.2  |
| 4.7.04  | 0.04 | 0.95 |
| 19.7.04 |      | 1.3  |
| 22.7.04 | 0.04 | 2.9  |

*Note.*—The obviously excessive dose of 0.1 milligramme was administered on 18.4.04 before it had been elicited by blood examination that the patient had not yet responded to the dose of 0.05 milligramme administered on 7.4.04. As a result—it may be assumed—of this cumulation of doses considerable constitutional disturbance was experienced. In view of this and of the development of a phlyctenule on the eye (a phenomenon which had once before been noticed in connection with the development of a negative phase) the inoculation fixed for the 28.4.04 was postponed and on the next occasion a smaller dose was administered.

A. A. ....

Patient, a young man, had been in bed for 12 months with a psoas abscess which discharged continuously and showed no signs of improvement. Treatment by inoculation was begun on April 8th. The patient is reported to have made rapid progress towards recovery immediately after the adoption of the treatment.

|         |       |     |
|---------|-------|-----|
| 8.4.04  | 0.002 | 0.4 |
| 20.4.04 | 0.002 |     |
| 3.5.04  |       | 0.5 |
| 10.5.04 | 0.004 |     |
| 31.5.04 | 0.01  |     |
| 2.6.04  |       | 0.8 |
| 16.6.04 |       | 1.1 |

M. O. ....

Patient, a young married woman, developed a double psoas abscess which was opened up before and behind, in January, 1903. The discharge from the wound continued till December without any sign of improvement, the temperature reaching 101° F. practically every night.

The treatment by inoculations of tubercle vaccine was begun in the middle of December, 1903, and has been continued since that date. The temperature became normal 10 days after the first inoculation, the wounds are practically healed and the patient is able to go out for drives. Her body weight has increased by 16 lbs.

Record of the inoculations which were carried out by the surgeon in charge of the case is not to hand.

|         |     |      |
|---------|-----|------|
| 10.6.04 | 0.1 | 1.95 |
| 19.6.04 |     |      |

S. T. ....

Patient, a man of 35, is the subject of phthisis associated with tubercular disease of the larynx.

|         |       |      |
|---------|-------|------|
| 2.6.04  | 0.002 | 0.6  |
| 8.6.04  |       | 1.15 |
| 9.6.04  | 0.004 |      |
| 21.6.04 |       | 0.98 |
| 22.6.04 | 0.008 |      |
| 12.7.04 | 0.008 | 0.84 |
| 25.7.04 |       | 1.86 |

Table III—*continued*.

| Initials.  | Brief history of case.  | Dates and particulars of inoculations undertaken since March, 1904.   | Opsonic power of the normal blood—A. E. W.'s—used as a control was taken as = 1). |
|------------|---|---|---|
| S. N. .... | Patient, a woman æt. 34, developed at age of 14 a tubercular abscess in left shoulder, at age of 16, a similar affection of the right leg behind knee, and at 17, lupus of the hand and foot. The little finger of the right hand was amputated in 1887, the third finger of the same hand in 1903, and three toes in April, 1904. In each case the tubercular infection has recurred in the site of the wound. | <p>in grm.</p> <p>13.6.04 0·004</p> <p>21.6.04 0·01</p> <p>27.6.04 0·005</p> <p>5.6.04</p> <p>14.7.04</p> <p>15.7.04 0·008</p> <p>21.7.04</p> | <p>0·9</p> <p>0·7</p> <p>0·65</p> <p>1·05</p> <p>0·73</p>                         |
| S. B. .... | Patient, a man of 22, has been the subject of lupus for the last 15 years. He has been treated by all the ordinary methods, including the application of Röntgen rays. Practically the whole of his face and neck are affected. He has also large patches on the scalp, trunk, and elbow of the right arm.  | <p>7.7.04 0·002</p> <p>11.7.04</p> <p>15.7.04 0·004</p> <p>18.7.04</p>  | <p>0·5</p> <p>0·44</p> <p>1·3</p>   |
| P. C. .... | Patient, a woman of 55, has for a long period of years been the subject of lupus of nose. The affection has now spread to her pharynx and larynx.   | <p>5.7.04 0·002</p> <p>12.7.04 0·004</p> <p>19.7.04</p>   | <p>0·56</p> <p>0·48</p> <p>1·0</p>  |
| J. S. .... | Patient, a man æt. 35, has suffered for two years from an inflammatory tumefaction of the subcutaneous tissues in the region of the angle of the jaw and anterior portion of the throat. The patient has been treated by scraping and the application of antiseptics.   | <p>4.7.04 1·0*</p> <p>5.7.04</p> <p>13.7.04 0·004†</p> <p>21.7.04</p>   | <p>0·67</p> <p>0·4</p> <p>0·76</p> <p>1·06</p>                                    |

\* Old tuberculin.

† New tuberculin.