

and therefore presumably of its own, a toxin which would cause necrosis in the mucous membrane of its own stomach were it not for some deterring influence, probably the concomitant formation of an anti-immune body.

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"On the Action exerted upon the *Staphylococcus pyogenes* by Human Blood Fluids, and on the Elaboration of Protective Elements in the Human Organism in Response to Inoculations of a *Staphylococcus* Vaccine." By A. E. WRIGHT, M.D., late Professor of Pathology, Army Medical School, Netley, Pathologist to St. Mary's Hospital, Paddington, W., 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.)

The subject matter with which we have here to deal may be distributed under the following headings:—

(1) Determination of the nature of the action which is exerted upon the *Staphylococcus pyogenes* by normal human blood fluids, and by the blood fluids of patients who have been inoculated with a staphylococcus vaccine.

(2) Comparison of the phagocytic power of the subjects of staphylococcus invasion with the phagocytic power of normal individuals.

(3) Distribution in the infected organism of the opsonins which here come into consideration.

(4) Determination of the question as to whether the opsonins are present in the blood of the infant at birth.

(5) Determination of the course of the reaction of immunisation which supervenes upon the inoculation of a staphylococcus vaccine.

(1) *Nature of the Action which is exerted upon the Staphylococcus pyogenes by Normal Blood Fluids, and by the Blood Fluids of Patients Inoculated with a Staphylococcus Vaccine.*

Bactericidal Action.—It was shown in the course of the classical researches on the bactericidal power of the blood which were conducted

by Nuttall* in Flügge's laboratory, that the staphylococcus offers resistance to the bactericidal action of the blood fluids. Two years afterwards the observations of Nuttall, which had reference to the blood of animals, were extended by Stern† to human blood. The methods employed by Nuttall and Stern alike did not, however, permit of a comparatively small bactericidal action being distinguished from a complete absence of bactericidal action.

The question as to how far the staphylococcus offers resistance to the bactericidal action of human blood was reinvestigated by one of us, the results being published (*a*) in a paper dealing with anti-staphylococcus inoculations‡, and (*b*) in a paper written in conjunction with Captain F. Windsor, I.M.S.,§ on the bactericidal action exerted by human blood upon a variety of pathogenic micro-organisms. It was established in the researches here in question, which were conducted with the more delicate methods of investigation set forth in the 'Proceedings of the Royal Society'¶ and in the 'Lancet'¶¶ respectively, (*a*) that normal human blood does not exert upon the staphylococcus any bactericidal action whatever, and (*b*) that anti-staphylococcus inoculations do not lead to a development of any bactericidal power in the blood.**

Opsonic Action.—It having become evident in the course of these researches that the effect of anti-staphylococcus inoculation is not to be found in a development of bactericidal properties in the blood fluids, attention was directed to the measurement of the phagocytic power of the blood. Taking to aid the method of phagocytic estimation devised by Major W. B. Leishman, R.A.M.C., it was ascertained that successful immunisation against staphylococcus goes in every case hand in hand with the acquirement of increased phagocytic power.

Certain difficulties having suggested themselves in connection with the attribution of this result to a "training" of the white blood corpuscles we addressed ourselves to a further investigation of the phenomena of phagocytosis.

In the course of this investigation†† it became clear that phagocytosis of bacteria is dependent upon an effect exerted upon the bacteria by the blood fluids. We spoke of this effect as an opsonic effect.

In a second research,‡‡ in which we extended our previous observations, on the opsonic power of the blood fluids, we showed that the increased

* Nuttall, 'Zeitschrift f. Hygiene,' 1888, vol. 4.

† Stern, 'Verhandlungen des IX Congresses f. Innere Medizin,' 1890.

‡ Wright, 'Lancet,' March 29, 1902.

§ Wright and Windsor, 'Journal of Hygiene,' vol. 2, No. 4, March, 1902.

¶ Wright, 'Roy. Soc. Proc.,' vol. 71, 1902.

¶¶ Wright, 'Lancet,' December 1, 1900, and March 2, 1901.

** *Vide* 'Journal of Hygiene' (*loc. cit.*), Tables VII and VIII.

†† Wright and Douglas, 'Roy. Soc. Proc.,' 1903, vol. 72.

‡‡ Wright and Douglas, 'Roy. Soc. Proc.,' 1904, vol. 73.

phagocytic effect which is obtained with the blood of successfully immunised persons is attributable not to any modification induced in the leucocytes, but to an increased opsonic power in the blood fluids. Conclusive evidence of this was obtained by separating, in the case of two bloods of conspicuously different phagocytic power in each case, the blood fluids from the corpuscular elements and then effecting an interchange of the blood fluids. The leucocytes of the successfully immunised patient exhibited under these circumstances the smaller phagocytic action characteristic of the blood of the normal individual who served as a control, while the leucocytes of the normal individual exhibited the increased phagocytic action characteristic of the blood of the successfully immunised patient.

The witness of the experiment just referred to, and of a previous experiment incorporated in our first paper, is confirmed by similar results obtained in connection with the tubercle bacillus. See pp. 164—165.

Agglutinating Action.—Normal human serum does not exert any characteristic agglutinating action upon the staphylococcus. Such agglutination as is obtained is not very sensibly increased under the influence of staphylococcus inoculations.

(2) *Comparison of the Phagocytic Power of the Subjects of Staphylococcus Invasion with the Phagocytic Power of Normal Persons.*

It is clear from what has been said above that the essential change which takes place in human blood, as a result of the inoculation of staphylococcus cultures, is an increase in the phagocytic power, dependent upon an increase of the opsonic elements in the blood.

Further evidence of the essential importance of the phagocytic and opsonic power in connection with resistance to staphylococcus invasions is obtained by contrasting the phagocytic power of the subjects of staphylococcus invasion with that of normal individuals.

Our observations on this subject were made in some instances by comparing the phagocytic power of the decalcified blood of the patient with the phagocytic power of the decalcified blood of a normal person. More frequently we employed in our experiments respectively, the patient's serum and the serum of a normal person in each case in association with the washed corpuscles derived from a normal man.

The results of our observations are tabulated below :—

Table showing the Ratio in which the Phagocytic or Opsonic Power of the Patient's Blood stood in each case to the Phagocytic or Opsonic Power of the Normal Individual who furnished the Control Blood.

(The phagocytic power of the control blood is taken in each case as unity.)

Initials of patient.	Form of staphylococcus invasion.	Phagocytic or opsonic index.
E. G.	Furunculosis	0·48
F. F.	Sycosis	0·49
J. E.	Acne	0·64
J. H.	Furunculosis	0·87
W. B.	Acne	0·55
E. H.	"	0·82
W. H.	Furunculosis	0·79
R. G.	"	0·7
G. L.	Acne and sycosis	0·74
S. C.	Furunculosis	0·87
W. L.	"	0·88
W. P.	"	0·39
S. F.	Very aggravated sycosis	0·1
E. F. D.	Acne	0·73
D. C.	Sycosis	0·8
J. M.	Acne	0·48
W. M.	Sycosis	0·37
E. P.	Acne	0·6
M. S.	Pustular affection of lips	0·6
F. V.	Repeated septic infection	0·47

In view of these observations and of the fact that we have not come across any instance of the association of a normal phagocytic power with a staphylococcus infection, the conclusion would seem justified that a low phagocytic power and staphylococcus infection are related to each other by some fact of causation. While it is *à priori* possible that the diminished phagocytic power which characterises those infected by the staphylococcus might be the result of the staphylococcus invasion, it is infinitely more probable, in view of the entire absence of clinical symptoms in the slighter cases of staphylococcus infection, that it is the defective phagocytic power of the patient which furnishes to the staphylococcus which is normally present upon the surface of the body the opportunity for invading the skin.

It is shown elsewhere (see p. 166) that a similar problem arises in connection with the circumstance that a low phagocytic power, with respect to the tubercle bacillus, is generally found in association with tubercular infection.

(3) *On the Distribution of Opsonins in the Infected Organism.*

It is a fundamentally important but unappreciated fact in connection with bacterial infections that the *bacteriotropic pressure*—we designate by this term the mass effect exerted upon the invading bacteria by the protective substances contained in the blood fluids—does not stand at the same level in every part of the infected organism.

One of us has, in conjunction with Captain George Lamb, I.M.S.,* demonstrated in the case of patients who had succumbed respectively to typhoid and Malta fever that the amount of agglutinins in the splenic pulp is invariably less;† in some instances over 200 times less, than in the circulating blood. It was further shown in the paper in question that there was a similar difference as between the fluid obtained from the typhoid spots and the fluid of the circulating blood. Captain Lamb‡ gave a further extension to these observations by demonstrating, in the case of monkeys examined immediately after the crisis of spirillum fever, that the splenic pulp (where the spirilla still survive after they have disappeared from the circulation) is much poorer in bactericidal and bacteriolytic substances than the circulating blood.

It is shown by these observations that the *Bacillus typhosus*, the *Micrococcus melitensis* and the *Spirillum Obermeyer*i, respectively multiply, or, as the case may be, maintain their existence, within the infected organism in regions of low bacteriotropic pressure. We may legitimately assume that the lowered bacteriotropic pressure in the nidus, where the micro-organisms are cultivating themselves, results from a retarded replacement of protective substances which are removed from the body fluids where these come into contact with bacteria.

Influenced by the results of the observations which have been just set out, we have addressed ourselves to the task of investigating the distribution of the opsonins in the case where the human organism is invaded by the staphylococcus. With this intent we have instituted comparisons between the serum obtained from the circulating blood and the fluid obtained by centrifugalisation from pus. It will be seen from the observations set forth below that what has been shown to hold true with respect to the distribution of agglutinins and bactericidal and bacteriolytic substances respectively in the bacterial infections before-mentioned, holds true also in the case of the opsonins in the case of staphylococcic infection. In view of this fact, and of the similar facts which we set out elsewhere in connection with tubercular infection (see pp. 167—169), it may be enunciated as a proposition of general

* Wright and Lamb, 'Lancet,' December 23, 1898.

† This observation so far as it applies to typhoid had been anticipated by Paul Courmont, 'Soc. de Biologie,' February 20 and March 28, 1897.

‡ Lamb, 'Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India,' vol. 12, pp. 96, *et seq.*

application that the invading micro-organisms cultivate themselves in the organism in regions of lowered bacteriostatic pressure.

Case 1.

13.4.04. Patient with an alveolar abscess pointing on the cheek. Pus gives a pure culture of staphylococcus.

A.

Patient's serum	2 vols.
A. E. W.'s washed corpuscles....	2 „
Staphylococcus emulsion	1 vol.

Phagocytic index* (average of 20 P.W.B.C.), 30·3.

B.

Supernatant fluid from pus....	2 vols.
A. E. W.'s washed corpuscles....	2 „
Staphylococcus emulsion	1 vol.

Phagocytic index (average of 20 P.W.B.C.), 5·1.

Ratio of opsonic index of serum to opsonic index of supernatant fluid from pus, 1 : 0·17.

15.4.04. Patient has had fomentations applied to cheek since abscess was opened on 13.4.04. Abscess rapidly healing.

A.

Patient's serum	2 vols.
A. E. W.'s washed corpuscles....	2 „
Staphylococcus emulsion	1 vol.

Phagocytic index (average of 20 P.W.B.C.), 10·05.

B.

Supernatant fluid from pus	2 vols.
A. E. W.'s washed corpuscles....	2 „
Staphylococcus emulsion	1 vol.

Phagocytic index (average of 20 P.W.B.C.), 10·1.

Ratio of opsonic index of serum to opsonic index of supernatant fluid of pus, 1 : 1.

Case 2.

Patient with patellar abscess. Pus from abscess furnishes a pure growth of streptococcus.

A.

Patient's serum	2 vols.
A. E. W.'s washed corpuscles....	2 „
Staphylococcus emulsion	1 vol.

Phagocytic index (average of 20 P.W.B.C.), 14·2.

* The phagocytic index was here, as elsewhere, determined by counting the number of bacteria ingested in the specified number of polynuclear leucocytes after digesting together in a capillary tube for 15 mins. at 37° C. the serum, corpuscles, and bacterial suspension.

B.

Supernatant fluid of pus. 2 vols.

A. E. W.'s washed corpuscles. . . 2 „

Staphylococcus emulsion 1 vol.

Phagocytic index (average of 40 P.W.B.C.), 1·25.

*Ratio of opsonic index of serum to opsonic index of supernatant fluid
of pus, 1 : 0·09.*

- (4) *Determination of the Question as to whether the Opsonins which come into Consideration in Connection with the Protection of the Organism against Staphylococcus Invasion are present in the Blood of the Infant at Birth.*

Opportunity offering, we have thought it worth while to determine whether the protective substances which come into consideration in connection with the Staphylococcus pyogenes are present in the blood at birth. For this purpose we have made a series of comparative estimations of the opsonic power of the blood of child and mother, employing for this purpose respectively placental blood and blood drawn off directly from the mother immediately after the completion of parturition. We are indebted to Messrs. B. H. Spilsbury and J. Freeman for the collection of the bloods. The observations we have made are as follows :—

Observations.

Blood drawn off, in the case of the mother, from the finger ; in the case of the child, from the umbilical cord.

No. 1.

A.

Mother's serum 3 vols.

A. E. W.'s washed corpuscles . . 3 „

Staphylococcus emulsion 1 vol.

Phagocytic index (average of 20 P.W.B.C.), 15·1.

B.

Child's serum. 3 vols.

A. E. W.'s washed corpuscles . . 3 „

Staphylococcus emulsion 1 vol.

Phagocytic index (average of 20 P.W.B.C.), 16·5.

No. 2.

A.

Mother's serum 2 vols.

A. E. W.'s washed corpuscles . . 2 „

Staphylococcus emulsion 1 vol.

Phagocytic index (average of 20 P.W.B.C.), 12·65.

B.

Child's serum. 2 vols.

A. E. W.'s washed corpuscles . . . 2 „

Staphylococcus emulsion 1 vol.

Phagocytic index (average of 20 P.W.B.C.), 12·25.

(5) *Determination of the Course of the Reaction of Immunisation obtained in response to Inoculations of a Staphylococcus Vaccine.*

We have in a very considerable number of cases plotted out by the aid of the phagocytic method the course of the reaction of immunisation which occurs in response to inoculations of a staphylococcus vaccine.

A preliminary word or two may be devoted to the description of the mode of preparation of the vaccine.

The procedure we adopt is as follows :—

We add to a 24 hours' growth of staphylococcus on sloped agar tube about 10 c.c. of sterile physiological salt solution. Churning up our culture with this, and letting it stand in order to allow all the unresolved bacterial masses to subside, we draw off the supernatant fluid by syphon action into a special form of tube and heat to 60° C. for half an hour.

We now place the tube in an incubator and incubate for 24 hours in order to allow of a multiplication of any bacteria which may have survived the heating. We now inoculate a sample of the heated culture upon agar with a view to the detection of any surviving micro-organisms ; at the same time, if this has not before been undertaken, we draw off a sample of the suspension and enumerate under the microscope by the procedure described by one of us in the 'Lancet' of July 5, 1902.

After verifying the sterility of the vaccine we now dilute with a sufficiency of physiological salt solution to bring down the number of staphylococci in the cubic centimetre to 2,500,000,000. Finally we add lysol in sufficient quantity to bring the content of the vaccine in this antiseptic to 0·25 per cent.

In connection with boils and sycosis a vaccine made from the *Staphylococcus aureus* ; in cases of acne a vaccine made from a mixture of *Staphylococcus albus* and *citreus* is appropriate.

A dose of 0·5 to 1 c.c. of the vaccine made as above is a suitable quantum for a first inoculation. For subsequent inoculations 1 to 2 c.c. of the vaccine may be employed.

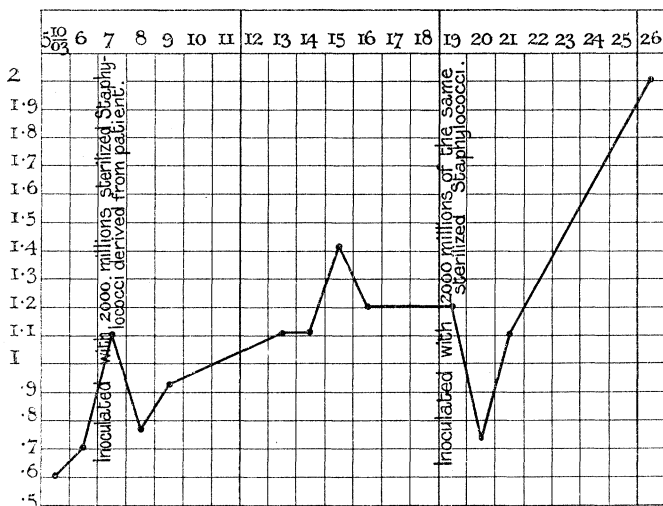
Below are subjoined four of the more instructive of the curves which we have obtained by the periodical examination of the phagocytic power of the blood subsequent to inoculations of staphylococcus vaccines.

Curve 1.—The curve here in question applies to a medical man who had suffered from boils almost continuously for 4 years.

On the date when he presented himself for treatment he had two boils on his neck. It will be seen that the phagocytic ratio recorded for that day was 0·6—the phagocytic power of the normal control blood being throughout taken as unity.

On the next day and the day subsequent phagocytic ratios of 0·7 and 1·1 respectively were recorded. This altogether spontaneous improvement of the phagocytic power went hand in hand with a striking improvement in the condition of the boils.

CURVE 1.



The patient was now inoculated with a quantum of sterilised culture of staphylococcus corresponding to 2,000,000,000 of staphylococci. The culture employed was derived from the patient's boils.

On the day subsequent to inoculation the patient's phagocytic power was found to be reduced. Contemporaneously with the development of this "negative phase," an irritable pimple developed on the neck. We may see in this, for it is a phenomenon which has manifested itself again and again in this connection with our inoculations, an indication that the negative phase is associated with a diminished resisting power to invasion by the staphylococcus.

On the second day after the inoculation an improvement in the phagocytic power was recorded. The "positive phase," which is here heralded, reached its acme on the eighth day subsequent to inoculation.

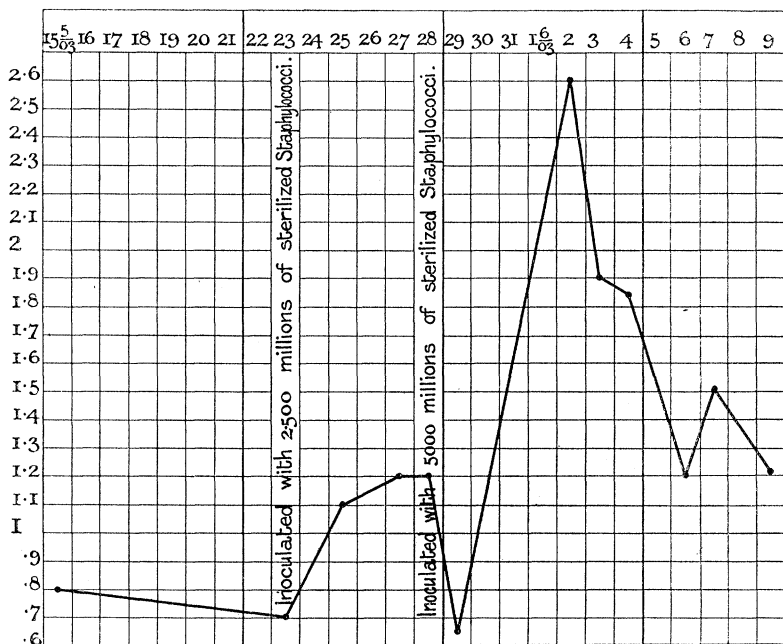
On the twelfth day the patient was re-inoculated with the same quantum of vaccine as was employed on the first occasion. As on the previous occasion, inoculation was followed by a negative, succeeded by a positive phase. For a period of weeks after the

inoculation, when the patient passed out of observation, he remained perfectly free from boils.

Curve 2.—This curve has reference to a patient who suffered from aggravated sycosis. A pure cultivation of *Staphylococcus citreus* was obtained from the inflamed hair follicles. He had been treated without appreciable benefit for 17 months by antiseptics.

Reference to the curve will show that the patient's phagocytic power with respect to the staphylococcus was here, as in the last case, less than that of the normal man who served as a control.

CURVE 2.

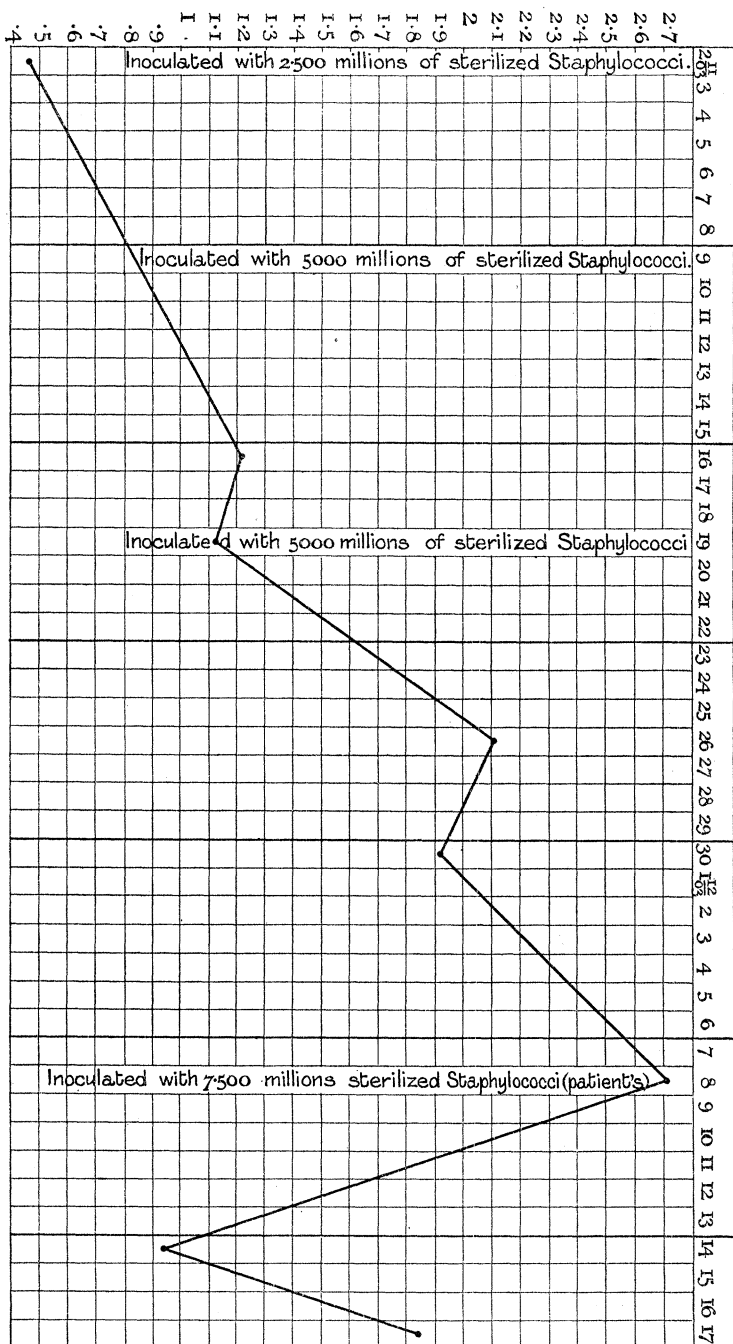


After his phagocytic power had been twice observed, he was inoculated with a quantum of sterilised staphylococcus culture corresponding to 2,500,000,000 of staphylococci. These staphylococci were derived from the culture above referred to.

Subsequent to inoculation we have here upon the curve instead of a rise preceded by a fall, only a rise. The absence of recorded negative phase is in all probability to be referred to the circumstance that 2 days here intervened between the inoculation and the first subsequent blood examination.

On the sixth day subsequent to inoculation the patient was re-inoculated with a double quantum of staphylococcus vaccine. This

CURVE 3.



inoculation was followed in a typical manner by a negative and positive phase. With respect to this last it will be seen that the curve attained its acme on the fifth day, and then declined in the usual manner.

Within a week after the second inoculation practically every trace of sycosis had disappeared. The patient was now lost sight of.

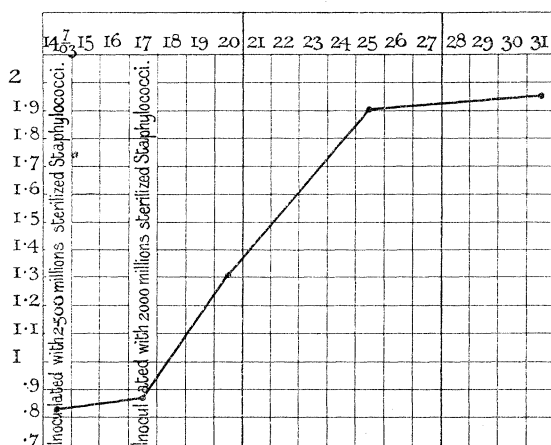
Curve 3.—The curve here in question applies to a labourer who was the subject of aggravated sycosis. He had suffered at intervals from childhood from boils and other chronic staphylococcus infections. A pure cultivation of *Staphylococcus aureus* was obtained from the inflamed hair follicles. He had been treated ineffectually for months by the usual methods.

As will be seen on reference to the chart, the phagocytic power of the blood was here investigated only from week to week instead of at more frequent intervals. As a result the positive phase of the reaction is alone on record in the case of the first three inoculations. In the case of the fourth inoculation—conducted with a larger quantum of the vaccine—the negative phase was still in evidence 6 days after the inoculation.

The patient, who was all but completely cured at the date upon which the curve concludes, afterwards relapsed after free indulgence in alcohol. He is now, as a result of further inoculations, again practically well.

Curve 4.—This curve applies to a healthy man of 24 who, while in training for a boat race, developed a boil on his gluteal region and subsequently a crop of boils on his neck.

CURVE 4.



Reference to the curve will show that his phagocytic index stood at the date of his first inoculation at 0.84.

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.

“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.