" Further Observations on the *Rôle* of the Blood Fluids in connection with Phagocytosis." By A. E. WRIGHT, M.D., late Professor of Pathology, Army Medical School, Netley, Pathologist to St. Mary's Hospital, W., and STEWART R. DOUGLAS, M.R.C.S., Captain, Indian Medical Service. Communicated by Sir J. BURDON SANDERSON, F.R.S. Received January 11,—Read February 25, 1904.

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

[PLATE 3.]

In a previous communication we showed that the phagocytosis which occurs when cultures of the Staphylococcus pyogenes are added to human blood, is directly dependent upon the presence of certain substances in the blood which exert a specific effect upon the bacteria. We suggested that the bacteriotropic substances here in question might appropriately be denoted by the term " opsonins."

In the present paper we propose to bring out certain further points in connection with the " opsonic power " of the blood.

RELATION OF THE OPSONIC POWER OF HUMAN BLOOD TO THE CAPACITY OF RESISTING INVASION BY THE STAPHYLOCOCCUS PYOGENES.

It has already been shown* by one of us that patients who are the subjects of acne, sycosis, or boils are characterised by a defective phagocytic power for the Staphylococcus pyogenes. We have recently been able to satisfy ourselves that this defective phagocytosis is dependent upon a defect of opsonic power.

It has also been shown by one of us that the cure of these bacterial infections, which can in almost every instance be achieved by the inoculation of appropriate quantities of sterilised staphylococcus cultures, is associated with the acquirement of an increased phagocytic power. We have now succeeded in establishing the fact—already adumbrated in our previous paper—that the increased phagocytosis which is associated with the achievement of the condition of immunisation here in question is dependent, not upon a modification of the white corpuscles, but upon a development of opsonins in the blood fluids.

The results of the subjoined experiment bring out this fact into clear relief.

Details of the Experiment.

Immunised Patient's Blood.—The patient, F. F., who had long been the subject of aggravated staphylococcic sycosis, had, after prolonged and

* 'Lancet,' March 29, 1902.

ineffectual treatment with antiseptics, been subjected to three successive inoculations of a sterilised staphylococcus culture. Under these inoculations his clinical condition had ameliorated itself in an astonishing manner, and his phagocytic power, which had previous to the date of inoculation been less by half than that of the normal man who served as a control, had increased in a progressive manner after each inoculation.

A sample of blood was now (by the technique elsewhere described)* drawn off and mixed with $\frac{1}{10}$th of its volume of 10 per cent. citrate of soda. A second sample of blood was drawn off and allowed to clot in the ordinary way.

In the case of the first sample of blood the corpuscles were isolated from the plasma by repeated washing with physiological salt solution, and centrifugalisation. The corpuscles thus isolated are referred to below as "washed corpuscles."

In the case of the second sample of blood the serum was simply separated from the corpuscles in the ordinary way by centrifugalisation.

Control Blood from a Normal Man.—The blood which served as a control was obtained from a normal healthy man. It was drawn off in exactly the same manner and was treated in each case by exactly the same procedures as the blood obtained from the patient.

Bacterial Culture.—The bacterial culture employed in the experiments set forth below was obtained by suspending in physiological salt solution a portion of a 24 hours' growth of Staphylococcus albus on agar.

The quantities of serum, washed corpuscles, and staphylococcus culture which are specified below were then in each case taken up into a capillary tube, mixed on a glass slide, re-aspirated into the tube, and digested together at blood heat for 15 minutes. Films were then made and stained by Leishman's stain. Finally the number of ingested bacteria were enumerated in a series of polynuclear W.B.C. taken in order as they came.

The phagocytic index given below—and the same applies throughout this paper—represents in each case the average number of bacteria ingested by the individual P.W.B.C. The number of polynuclear white blood corpuscles which have furnished the index is in each case inserted in brackets :—

Experiment.

A.

Immunised patient's washed corpuscles	3 vols.
Immunised patient's serum	3 ,,
Suspension of staphylococcus culture	1 vol.

Phagocytic index (20 P.W.B.C.), 25·7.

* 'Lancet,' January 23, 1904.

B.

Washed corpuscles from normal man	3 vols.
Serum from normal man	3 „
Suspension of staphylococcus culture	1 vol.

Phagocytic index (15 P.W.B.C.), 13.

C.

Immunised patient's washed corpuscles	3 vols.
Serum from normal man	3 „
Suspension of staphylococcus culture	1 vol.

Phagocytic index (15 P.W.B.C.), 13.

D.

Washed corpuscles from normal man	3 vols.
Serum from immunised patient	3 „
Suspension of staphylococcus culture	1 vol.

Phagocytic index (15 P.W.B.C.), 28·2.

Experiments on the Opsonic Power of Human Blood in its Relation to the Bacillus of Plague.

In these and all subsequent experiments, unless where otherwise specified, the technique employed was exactly the same as that employed in the experiments set forth above. It may further be premised that the bacterial suspensions employed were in each case suspensions of very young agar cultures—in most cases 24-hour cultures—in physiological salt solution. By the term "heated serum" is in each case to be understood serum which has been subjected to a temperature of 60° C. for 10 minutes or more.

Experiment 1.

A.

S. R. D.'s unheated serum.....	3 vols.
S. R. D.'s washed corpuscles.....	3 „
Suspension of plague bacillus	1 vol.

Phagocytic index (20 P.W.B.C.), 3·0.

B.

S. R. D.'s heated serum	3 vols.
S. R. D.'s washed corpuscles.....	3 „
Suspension of plague bacillus	1 vol.

Phagocytic index (25 P.W.B.C.), 0·7.

Experiment 2.

A.

S. R. D.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of plague bacillus	1 vol.

Phagocytic index (20 P.W.B.C.), 13·1.

B.

S. R. D.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of plague bacillus	1 vol.

Phagocytic index (20 P.W.B.C.), 2·1.

Experiment 3.

A.

A. E. W.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of plague bacillus	1 vol.

Phagocytic index (21 P.W.B.C.), 19·6.

B.

A. E. W.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of plague bacillus	1 vol

Phagocytic index (54 P.W.B.C.), 8·4.

Experiment 4.

A.

B. H. S.'s unheated serum	2 vols.
A. E. W.'s washed corpuscles	2 „
Suspension of plague bacillus	1 vol.

Phagocytic index (43 P.W.B.C.), 5·3.

B.

B. H. S.'s heated serum	2 vols.
A. E. W.'s washed corpuscles	2 „
Suspension of plague bacillus	1 vol.

Phagocytic index (43 P.W.B.C.), 1·4.

It may incidentally be noted in connection with these experiments that while the plague bacilli which lay free in the films were in each case quite unaltered, many of those which had been ingested showed

extremely characteristic involution forms* such as we have not seen since we worked with freshly isolated plague cultures in Bombay in connection with the Indian Plague Commission. So typical were the involution forms of the ingested plague bacilli, that we should not hesitate to employ the method of phagocytosis as an aid to diagnosis in the case of a doubtful plague culture.

Experiments on the Opsonic Power of Human Blood in Relation to Micrococcus Melitensis.

Experiment 1.

A.

S. R. D.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of *Micrococcus Melitensis*	1 „

Phagocytic index (10 P.W.B.C.), 26·9.

B.

S. R. D.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of *Micrococcus Melitensis*	1 „

Phagocytic index (10 P.W.B.C.), 9·2.

Experiment 2.

A.

A. E. W.'s unheated serum	3 vols.
A. E. W.'s washed corpuscles	3 „
Suspension of *Micrococcus Melitensis*	1 „

Phagocytic index (21 P.W.B.C.), 10·0.

B.

A. E. W.'s heated serum	3 vols.
A. E. W.'s washed corpuscles	3 „
Suspension of *Micrococcus Melitensis*	1 vol.

Phagocytic index (21 P.W.B.C.), 2·4.

Experiment 3.

A.

S. R. D.'s heated serum	3 vols.
A. E. W.'s washed corpuscles	3 „
Suspension of *Micrococcus Melitensis*	1 vol.

Phagocytic index (21 P.W.B.C.), 12·9.

* It may be observed that our plague culture—like other plague cultures which have been cultivated on artificial nutrient media for a number of generations—has altogether lost the property of developing in a spontaneous manner the involution forms which are characteristic of freshly isolated plague cultures.

B.

S. R. D.'s heated serum	3 vols.
A. E. W.'s washed corpuscles	3 „
Suspension of *Micrococcus Melitensis*	1 vol.

Phagocytic index (21 P.W.B.C.), 0·9.

Experiments on the Opsonic Power of Human Blood in Relation to the Bacillus dysentericus (Shiga).

Experiment 1.

A.

S. R. D.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of Shiga's bacillus	1 vol.

Phagocytic index (20 W.P.B.C.), 4·2.

B.

S. R. D.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of Shiga's bacillus	1 vol.

Phagocytic index (20 P.W.B.C.), 0·0.

Experiment 2.

A.

A. E. W.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of Shiga's bacillus	1 vol.

Phagocytic index (20 P.W.B.C.), 5·4.

B.

A. E. W.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of Shiga's bacillus	1 vol.

Phagocytic index (33 P.W.B.C.), 0·1.

Experiment 3.

A.

S. R. D.'s unheated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Suspension of Shiga's bacillus	1 vol.

Phagocytic index (20 P.W.B.C.), 3·6

B.

S. R. D.'s heated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Suspension of Shiga's bacillus	1 vol.

Phagocytic index (20 P.W.B.C.), 0·2.

A certain number of the bacilli (and these bacilli were found indifferently in the interior of the cells and free in the preparation) had, in the case of the experiments undertaken with unheated serum, undergone spherulation.

Experiments on the Opsonic Power of Human Blood in its Relation to the Bacillus coli.

Experiment 1.

A.

B. H. S.'s unheated serum	3 vols.
B. H. S.'s washed corpuscles	3 „
Suspension of the *Bacillus coli*	1 vol.

Phagocytic index (20 P.W.B.C.), 3·8.

B.

B. H. S.'s heated serum	3 vols.
B. H. S.'s washed corpuscles	3 „
Suspension of the *Bacillus coli*	1 vol.

Phagocytic index (20 P.W.B.C.), 0·75.

Experiment 2.

A.

F. F.'s unheated serum	3 vols.
F. F.'s washed corpuscles	3 „
Suspension of the *Bacillus coli*	1 vol.

Phagocytic index (20 P.W.B.C.), 5.

B.

F. F.'s heated serum	3 vols.
F. F.'s washed corpuscles	3 „
Suspension of the *Bacillus coli*	1 vol.

Phagocytic index (21 P.W.B.C.), 0·76.

Experiments on the Opsonic Power of Human Blood in its Relation to the Pneumococcus of Fraenkel.

Experiment 1.

A.

S. R. D.'s unheated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Suspension of the pneumococcus of Fraenkel	1 vol.

Phagocytic index (15 P.W.B.C.), 16.

B.

S. R. D.'s heated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Suspension of Fraenkel's pneumococcus	1 vol.

Phagocytic index (40 P.W.B.C.), 1·1.

Experiment 2.

A.

A. E. W.'s unheated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Suspension of Fraenkel's pneumococcus	1 vol.

Phagocytic index (23 P.W.B.C.), 6.

B.

A. E. W.'s heated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Suspension of Fraenkel's pneumococcus	1 vol.

Phagocytic index (40 P.W.B.C.), 0·2.

Experiments on the Opsonic Power of Human Blood in its Relation to the Bacillus of Anthrax.

Experiment 1.

A.

S. R. D.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of *Bacillus anthracis*	1 vol.

Enumeration was here impossible, but there was everywhere evidence of phagocytosis. In the few cases where the leucocytes had not ingested bacteria, they were found to have extended themselves in a characteristic grasping manner along the bacterial threads (fig. 5).

B.

S. R. D.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of the *Bacillus anthracis*	1 vol.

Here there were practically no signs of phagocytosis. The cells were everywhere empty, and they had not drawn themselves into intimate contact with the anthrax threads (fig. 6).

Experiment 2.

A.

A. E. W.'s unheated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Broth culture of anthrax	1 vol.

Phagocytic index (36 P.W.B.C.), 2·4 (approximate only).

B.

A. E. W.'s heated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Broth culture of anthrax	1 vol.

Phagocytic index (100 P.W.B.C.), 0.

Opsonic Power of Human Blood in its Relation to the Bacillus typhosus and the Cholera Vibrio.

It is well known that human blood exerts a very considerable bactericidal power upon cultures of the *Bacillus typhosus* and of the cholera vibrio. The destructive effect in question manifests itself to microscopical observation in the form of very profound morphological changes which come under observation in cultures which have been digested with unheated serum. The bacteria in such cultures, after undergoing agglutination and spherulation, swell up and lose their chemical affinity for anilin dyes. Finally they are completely dissolved.

It is manifest that where disintegrative changes of this kind are occurring under the influence of the serum, opsonic effects will be more or less thrust into the background. These last will, in the case of phagocytic experiments conducted with unheated serum, be masked, on the one hand, by the fact that there will be fewer bacteria available for phagocytosis, and on the other hand by the fact that intracellular disintegration will, it may be presumed, be more rapid in the case where the serum has already exerted a disintegrative effect on the bacteria anterior to their ingestion.

Lastly, ingested bacteria which have lost their characteristic chemical affinity for their stain may readily escape enumeration.

All these points must be taken into consideration in connection with the subjoined experiments:—

Experiment 1.

A.

S. R. D.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of the cholera vibrio	2 „

Everywhere considerable phagocytosis. Complete spherulation of almost all the micro-organisms within and all the micro-organisms outside the cells. No indication of vacuolation round the ingested bacteria (fig. 3).

Phagocytic index (14 P.W.B.C.), 24 (*circ.*).

B.

S. R. D.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of the cholera vibrio	2 „

Everywhere considerable phagocytosis. No spherulation of the micro-organisms either within or without the leucocytes. Very marked vacuolation of the leucocytes round the ingested bacteria (fig. 4).

Phagocytic index (11 P.W.B.C.), 26·2 (*circ.*).

Experiment 2.

A.

A. E. W.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of the cholera vibrio	1 vol.

Complete spherulation of all the bacteria, whether within or without the cells.

Phagocytic index (21 P.W.B.C.), 8·1 (*circ.*).

B.

A. E. W.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of the cholera vibrio	1 vol.

No spherulation of the micro-organisms, either within or without the leucocytes.

Phagocytic index (13 P.W.B.C.), 0·8.

Experiment 3.

A.

S. R. D.'s unheated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Broth culture of the typhoid bacillus	2 „

Much phagocytosis. Complete spherulation of all the extracellular micro-organisms. Many of the bacilli in the interior of the leucocytes have completely preserved their original contours, others—probably the later ingested ones—are spherulated (fig. 1).

B.

S. R. D.'s heated serum	2 vols.
S. R. D.'s washed corpuscles	2 „
Broth culture of the typhoid bacillus	2 „

Much phagocytosis. All the micro-organisms, whether within or without the leucocytes, are morphologically unaltered and have preserved their staining properties unimpaired (fig. 2.).

Experiment 4.

A.

A. E. W.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Broth cultivation of the typhoid bacillus	2 „

Complete spherulation of all the extracellular bacteria which have escaped solution. In interior of leucocytes most of the bacteria have undergone spherulation, but in the centre of the corpuscles some—probably those which were soonest ingested—are morphologically unaltered and preserve their staining properties unaltered.

Phagocytic index, 100 (estimated).

B.

A. E. W.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Broth cultivation of the typhoid bacillus	1 vol.

No spherulation, either within or without the cells.

Phagocytic index (20 P.W.B.C.), 31·8 (*circ.*).

Experiment 5.

A.

S. R. D.'s unheated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of the typhoid bacillus	1 vol.

All the bacilli both within and without the cells have undergone spherulation.

Phagocytic index (11 P.W.B.C.), 13·6.

B.

S. R. D.'s heated serum	3 vols.
S. R. D.'s washed corpuscles	3 „
Suspension of the typhoid bacillus	1 vol.

No spherulation either within or without the leucocytes.

Phagocytic index (23 P.W.B.C.), 7·2.

Of incidental interest in connection with the above experiments is the demonstration which they afford, that the spherulation of the intracellular ingested micro-organisms, which has been often ascribed to the agency of the leucocytes, is in reality due to agency of the blood fluids.

Opsonic Power of Human Blood in its Relation to the Diphtheria Bacillus and the Xerosis Bacillus.

Experiment 1.

A.

A. E. W.'s unheated serum	3 vols.
A. E. W.'s washed corpuscles	3 „
Suspension of the diphtheria bacillus	3 „

Phagocytic index (27 P.W.B.C.), 0·7.

B.

A. E. W.'s heated serum	3 vols.
A. E. W.'s washed corpuscles	3 „
Suspension of the diphtheria bacillus	3 „

Phagocytic index (29 P.W.B.C.), 4·1.

Experiment 2.

A.

B. H. S.'s unheated serum	3 vols.
B. H. S.'s washed corpuscles	3 „
Suspension of the diphtheria bacillus	2 „

Phagocytic index (20 P.W.B.C.), 8·0.

B.

B. H. S.'s heated serum	3 vols.
B. H. S.'s washed corpuscles	3 „
Suspension of the diphtheria bacillus	2 „

Phagocytic index (20 P.W.B.C.), 10·9.

Experiment 3.

A.

B. H. S.'s unheated serum	3 vols.
B. H. S.'s washed corpuscle s	3 ,,
Suspension of the diphtheria bacillus	1 vol.

Phagocytic index (44 P.W.B.C.), 4·0.

B.

B. H. S.'s heated serum	3 vols.
B. H. S.'s washed corpuscles	3 ,,
Suspension of the diphtheria bacillus	1 vol.

Phagocytic index (50 P.W.B.C.), 3·3.

Experiment 4.

A.

A. E. W.'s unheated serum	3 vols.
A. E. W.'s washed corpuscles	3 ,,
Suspension of the xerosis bacillus	1 vol.

Phagocytic index (40 P.W.B.C.), 2·8.

B.

A. E. W.'s heated serum	3 vols.
A. E. W.'s washed corpuscles	3 ,,
Suspension of the xerosis bacillus	1 vol.

Phagocytic index (25 P.W.B.C.), 3·2.

Experiment 5.

A.

B. H. S.'s unheated serum	3 vols.
B. H. S.'s washed corpuscles	3 ,,
Suspension of the xerosis bacillus	1 vol.

Phagocytic index (30 P.W.B.C.), 6·3.

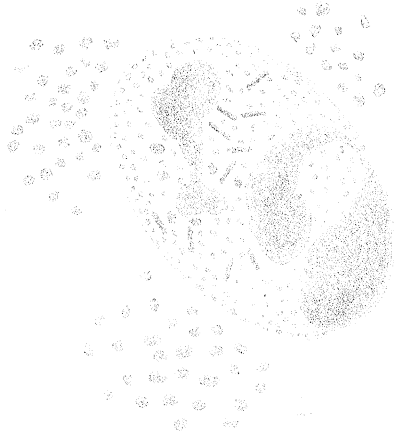
B.

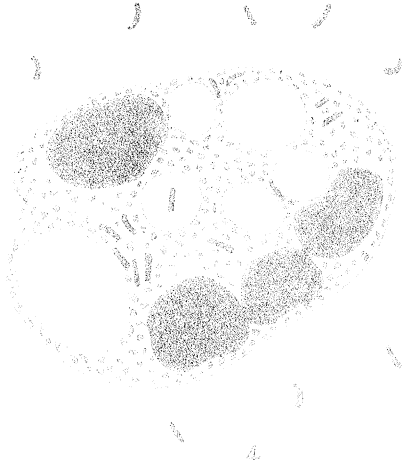
B. H. S.'s heated serum	3 vols.
B. H. S.'s washed corpuscles	3 ,,
Suspension of the xerosis bacillus	1 vol.

Phagocytic index (30 P.W.B.C.), 6.

Conclusions.

The experimental data which have been set forth above establish that the opsonic action of the blood fluids—to which attention was for the first time directed in our previous communication—is exerted not exclusively upon the *Staphylococcus pyogenes*, but also upon the *Bacillus*





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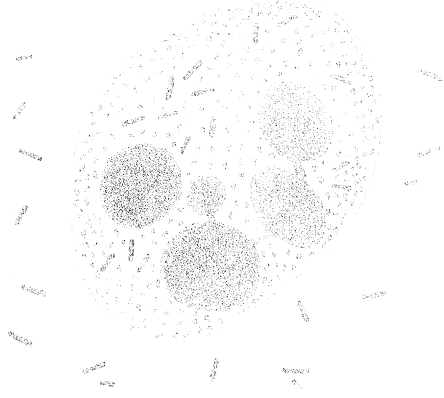

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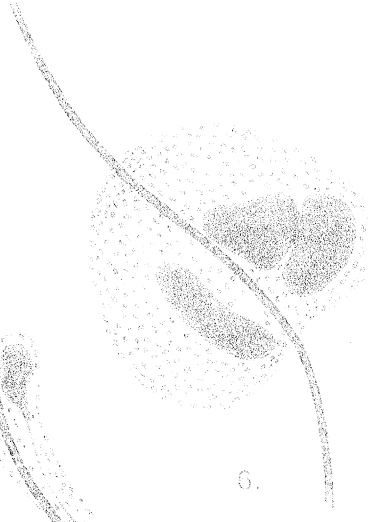

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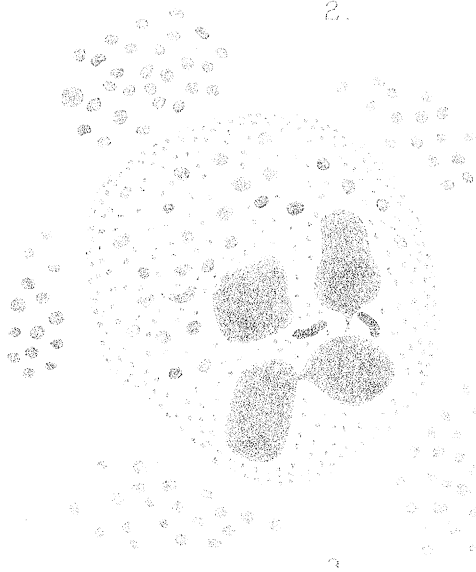

3.

5.

pestis, the *Micrococcus melitensis*, the *Diplococcus pneumoniæ* of Fraenkel, the *Bacillus coli*, the *Bacillus dysenteriæ* (Shiga), the *Bacillus anthracis*, the *Bacillus typhosus*, and the *Vibrio choleræ Asiaticæ*.

So far as we have gone, the *Bacillus diphtheriæ* and its congener the *Bacillus xerosis* have proved to be the only pathogenetic bacteria which are insensible to this action of the blood fluids.

Taking these experimental data in conjunction with other facts which have been elicited by us, or as the case may be by one of us working in connection with Captain F. Windsor,* I.M.S., with regard to the bactericidal action exerted by human blood upon the various species of pathogenetic micro-organisms, we may classify these bacteria in the following categories:—

(1) *Bacteria which are eminently sensible to the bactericidal, bacteriolytic, and opsonic action of normal human blood fluids.*—The *Bacillus typhosus* and the *Vibrio choleræ Asiaticæ*.

(2) *Bacteria which are in some measure sensible to the bactericidal action of the normal human blood fluids, and which are eminently sensible to its opsonic action.*—The *Bacillus coli* and the *Bacillus dysenteriæ*.

(3) *Bacteria which are absolutely insensible to the bactericidal action of the normal human blood fluids, but are eminently sensible to the opsonic action of these fluids.*—The *Staphylococcus pyogenes*, the *Bacillus pestis*, the *Micrococcus melitensis*, the *Diplococcus pneumoniæ* of Fraenkel.

(4) *Bacteria which are insensible both to the bactericidal and to the opsonic action of human blood fluids.*—The *Bacillus diphtheriæ* and *Bacillus xerosis*.

It may be pointed out in conclusion that the demonstration furnished above, that successful immunisation against the staphylococcus pyogenes is dependent upon an elaboration of opsonins in the system of the inoculated patient, suggests that successful immunisation against plague and Malta fever, and we may add against streptococcal invasions, may be likewise dependent upon the elaboration of opsonins.

It will be manifest that if this is so, the determination of the opsonic power of the blood is calculated to render services also in connection with the testing of any therapeutic sera which may find an application in connection with the disease.

DESCRIPTION OF PLATE 3.

FIG. 1.—White Blood Corpuscles digested with *unheated* serum and culture of the *Bacillus typhosus* for 15 minutes at 37° C. Shows, in the case of the extra-cellular micro-organisms, complete spherulation and agglutination. Many of the micro-organisms in the interior of the phagocyte are unaltered with respect to their shape and staining reaction; others—presumably the later ingested micro-organisms—have undergone spherulation.

* 'Journ. of Hygiene,' vol. 1.

FIG. 2.—White Blood Corpuscles digested with *heated* serum and culture of the *Bacillus typhosus* for 15 minutes at 37° C. Shows that the micro-organisms retain their shape, both within and without the phagocyte.

FIG. 3.—White Blood Corpuscles digested with *unheated* serum and culture of the Cholera Vibrio for 15 minutes at 37° C. Shows, in the case of the extra-cellular micro-organisms, complete agglutination and spherulation. Two of the micro-organisms in the interior of the phagocyte—presumably those first ingested—retain their characteristic shape.

FIG. 4.—White Blood Corpuscles digested with *heated* serum and culture of the Cholera Vibrio for 15 minutes at 37° C. Shows vacuolation of the phagocyte and no alteration in the micro-organisms, either within or without the phagocyte.

FIG. 5.—White Blood Corpuscles digested with *unheated* serum and culture of the *Bacillus anthracis* for 15 minutes at 37° C. Shows the phagocyte extending itself in such a manner as to invaginate the bacilli.

FIG. 6.—White Blood Corpuscles digested with *heated* serum and culture of the *Bacillus anthracis*. Shows an anthrax thread lying upon a phagocyte, which makes no attempt at phagocytosis.

"Sunspot Variation in Latitude, 1861—1902." By WILLIAM J. S. LOCKYER, M.A. (Camb.), Ph.D. (Gött.), F.R.A.S., Chief Assistant, Solar Physics Observatory. Communicated by Sir NORMAN LOCKYER, K.C.B., LL.D., F.R.S. Received January 16, —Read February 11, 1904.

[PLATES 4 AND 5.]

In a previous communication,* Sir Norman Lockyer and I gave the results of a discussion of prominence observations, and pointed out the necessity of dealing individually with small zones on the solar surface.

In that paper brief reference was made to the law of spot zones, as discovered by Carrington, and corroborated by Spörer, and it was further stated that more modern observations had established these *general* deductions of spot distribution. The words "general deductions" were purposely used, as it was then noticed that there were many anomalies that required explanation.

The object of the present paper is to draw attention to these anomalies, and to give the results that have been deduced from a minute examination of the changes of heliographic latitudes of sunspots from year to year. The present evidence indicates that the law of Spörer, although of great importance, represents only a very general idea of a complicated sunspot circulation.

* "Solar Prominence and Spot Circulation, 1872—1901," 'Roy. Soc. Proc.,' vol. 71, p. 446.

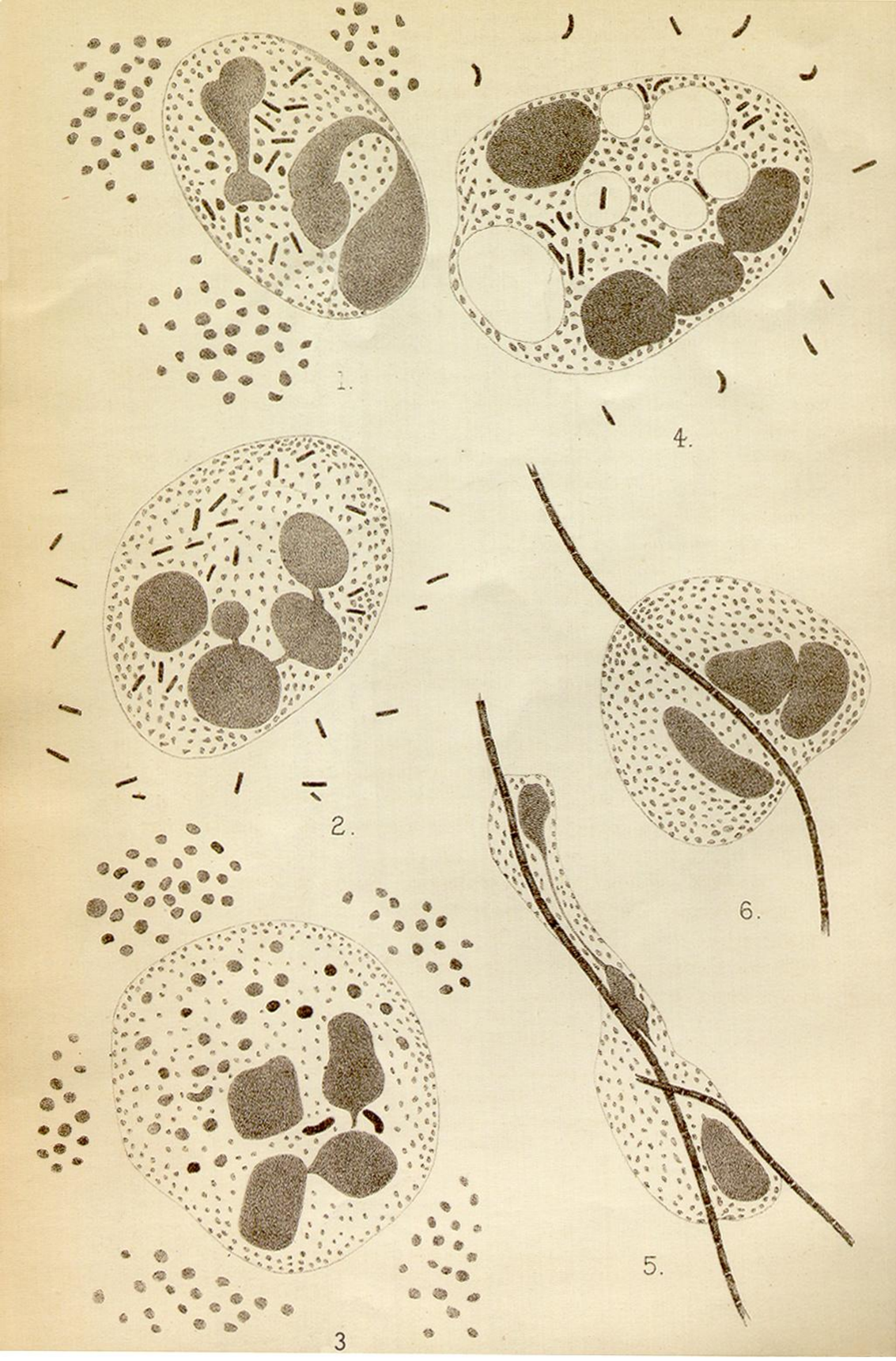

FIG. 1.—White Blood Corpuscles digested with *unheated* serum and culture of the *Bacillus typhosus* for 15 minutes at 37° C. Shows, in the case of the extra-cellular micro-organisms, complete spherulation and agglutination. Many of the micro-organisms in the interior of the phagocyte are unaltered with respect to their shape and staining reaction; others—presumably the later ingested micro-organisms—have undergone spherulation.

FIG. 2.—White Blood Corpuscles digested with *heated* serum and culture of the *Bacillus typhosus* for 15 minutes at 37° C. Shows that the micro-organisms retain their shape, both within and without the phagocyte.

FIG. 3.—White Blood Corpuscles digested with *unheated* serum and culture of the Cholera Vibrio for 15 minutes at 37° C. Shows, in the case of the extra-cellular micro-organisms, complete agglutination and spherulation. Two of the micro-organisms in the interior of the phagocyte—presumably those first ingested—retain their characteristic shape.

FIG. 4.—White Blood Corpuscles digested with *heated* serum and culture of the Cholera Vibrio for 15 minutes at 37° C. Shows vacuolation of the phagocyte and no alteration in the micro-organisms, either within or without the phagocyte.

FIG. 5.—White Blood Corpuscles digested with *unheated* serum and culture of the *Bacillus anthracis* for 15 minutes at 37° C. Shows the phagocyte extending itself in such a manner as to invaginate the bacilli.

FIG. 6.—White Blood Corpuscles digested with *heated* serum and culture of the *Bacillus anthracis*. Shows an anthrax thread lying upon a phagocyte, which makes no attempt at phagocytosis.