EXPLANATION OF PLATE No. 6.

All figures drawn with Abbé camera lucida, using 2 mm. apochromatic objective and 12 compensating ocular (Zeiss). Magnification 1800 diameters approximately.

Figs. 1–20.—Rhodesian strain of human trypanosome. Figures drawn from parasites in the blood of rats, except where otherwise stated.

Fig. 1.—Stout form with nucleus median.
Figs. 2–8.—Stout and stumpy forms, each with posterior nucleus. The nucleus is seen gradually to become more posterior, till it lies behind the blepharoplast (fig. 9).
Fig. 10.—Posterior nuclear form with line connecting blepharoplast and nucleus.
Fig. 11.—Posterior nuclear form becoming rounded.
Fig. 12.—Posterior nuclear form showing division.
Figs. 13–17.—"Snout" forms. (Figs. 13, 15 from rat's blood, figs. 14, 16, 17 from man.)
Figs. 18, 19.—Forms with terminal blepharoplast.
Fig. 20.—Multiple division form, with four blepharoplasts and two nuclei. Such parasites are not uncommon in the Rhodesian strain.

Figs. 21–25.—Various trypanosomes drawn from the blood of rats infected with the old laboratory strain of *T. gambiense*.

The Influence of Bacterial Endotoxins on Phagocytosis, including a New Method for the Differentiation of Bacteria.

(Second Report.)

By Leonard S. Dudgeon, P. N. Panton and H. A. F. Wilson.

(Communicated by Dr. F. W. Mott, F.R.S. Received August 2,—Read November 17, 1910.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

It was shown in a paper communicated by us to the Royal Society in April, 1910—

(*a*) That bacterial endotoxins have the power of inhibiting phagocytosis; that in some cases this action is general, but in most cases it is specific.

(*b*) That the endotoxic substance is unaltered by prolonged exposure to high temperatures.

(*c*) That, as far as our experiments then carried us, the inhibition of phagocytosis appeared to result from an interaction between endotoxin and serum.

The further investigations which form the basis of this communication have been mainly directed towards the elucidation of the mode of action of the endotoxic substance. Firstly, whether it acts on the serum, the

leucocytes, or the bacteria; secondly, how far this action is specific for various organisms belonging to the same family; and thirdly, whether the inhibition of phagocytosis is due to absorption of complement by the endotoxin.

In the present series of experiments the technique adopted has been the same as described in the preliminary report, with the exception that the bacteria were allowed to grow for a longer period of time, so as to obtain a more abundant growth for the preparation of the endotoxin. In every case the bacterial deposit was alternately frozen by means of solid carbon dioxide and thawed, as by this method a more potent extract was obtained. For each group of experiments a fresh extract was prepared.

Is the action of the Endotoxic Substance on the Leucocytes?

Equal parts of the extract and washed human leucocytes were mixed together and incubated for one hour at 37° C.; they were then centrifugalised and the supernatant fluid pipetted off. One volume of the treated leucocytes was added to equal volumes of serum and bacillary suspension and incubated for 15 minutes at 37° C. In every instance the leucocytes were found to be unaffected, since as marked phagocytosis occurred when the leucocytes had been submitted to this treatment as in the control. The following brief experiment will serve to illustrate this point:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. (Normal leucocytes + saline) + normal serum + *B. Achard*	328	1
„ „ + „ + *B. typhosus*	132	4
B. (Normal leucocytes + Achard extract) + normal serum + *B. Achard*	300	2
„ „ „ + „ + *B. typhosus*	138	4
C. (Normal leucocytes + typhoid extract) + normal serum + *B. Achard*	369	0
„ „ „ + „ + *B. typhosus*	156	8

Is the action of the Endotoxic Substance on the Bacteria themselves?

For this series of experiments a given volume of extract was added to an equal volume of bacteria and incubated for one hour at 37° C. The mixture was then centrifugalised at very high speed until a clear supernatant fluid was obtained; this was pipetted off and an equal volume of normal saline was added to the deposit and the whole thoroughly mixed. A volume of this final bacterial mixture was then added to equal volumes of normal serum and leucocytes. The result of these experiments was to show that no action took place between the organisms and the endotoxic substance, and that

phagocytosis was unaffected. It is only necessary to cite one experiment, as all our results were identical:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Normal saline + *B. coli* + normal leucocytes + normal serum.....	361	0
„ + *B. Danysz* + normal leucocytes + normal serum	313	1
B. Coli extract + *B. coli* + normal leucocytes + normal serum	420	0
„ + *B. Danysz* + normal leucocytes + normal serum ...	339	0
C. Danysz extract + *B. coli* + „ + „ ...	449	0
„ + *B. Danysz* + normal leucocytes + normal serum	385	0

Is the action of the Endotoxic Substance on the Serum?

As it was evident that the action of this body was not directly related to the leucocytes or the organisms themselves, further investigations, mostly of a confirmatory nature, were undertaken in connection with its action on the serum. The same technique has been employed as given in detail in our previous report.

Numerous experiments were made for the purpose of ascertaining whether absorption of complement and inhibition of phagocytosis, resulting from the action of endotoxin upon normal serum, were directly related. It was found, however, that no greater phagocytosis occurred in those instances where ample hæmolytic complement was still present at the end of the period of interaction than if all the complement had been absorbed by the endotoxic substance. For the hæmolytic experiments either sheep's red cells or sensitised human red cells were employed. The following experiment may be cited to illustrate these points:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.	Hæmolytic experiments.
A. Serum saline* + leucocytes + *B. typhosus*	100	8	Complete hæmolysis.
„ „ + „ + *B. paratyphoid*	118	3	
B. Normal serum paratyphoid extract + leucocytes + *B. typhosus* ...	65	16	No hæmolysis.
„ „ „ + leucocytes + *B. paratyphoid*	16	40	
C. Normal serum typhoid extract + leucocytes + *B. typhosus*	8	43	No hæmolysis.
„ „ „ + „ + *B. paratyphoid*	90	7	

* By "serum saline," "serum paratyphoid extract," etc., is meant one volume of a mixture of equal parts of serum and saline, or of serum and the bacterial extract in question, after one hour's incubation at 37° C.

Experiment C of this series serves to show that, while the hæmolytic complement was completely absorbed by the endotoxin, the degree of phagocytosis of the foreign organism was little altered.

Specificity.

In the experiment referred to below, the specific action of the endotoxin is clearly shown. We have already explained at the commencement of this paper that when the endotoxin and leucocytes are incubated together there is no reduction in the amount of phagocytic activity as occurs here.

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Serum saline + leucocytes + *B. Achard*	328	1
„ + „ + *B. typhosus*	132	4
B. Serum Achard extract + leucocytes + *B. Achard*.....	40	26
„ „ + „ + *B. typhosus*.....	176	5
C. Serum typhoid extract + leucocytes + *B. Achard*	155	6
„ „ „ + „ + *B. typhosus*	0	50

The specific nature of this reaction, even between such closely allied organisms as *B. typhosus* and *B. Achard*, is thus proved beyond question. Similar results were obtained with many other members of the typhoid-coli-paratyphoid family. Further, experiments were made to determine whether organisms of the same species, but derived from different sources, would yield an endotoxin with identical properties or not.

In the following instance two strains of *B. typhosus* were used. One strain was isolated from a gall-bladder and has been frequently sub-cultured for the last four years, while the other was recently isolated from the blood of a patient suffering from typhoid fever:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Serum saline + leucocytes + *B. typhosus* (bile)	219	1
„ + „ + *B. typhosus*	126	5
B. Serum typhoid extract + leucocytes + *B. typhosus* (bile)	3	48
„ „ + „ + *B. typhosus*	7	46
C. Serum typhoid (bile) extract + leucocytes + *B. typhosus* (bile) ...	5	45
„ „ + „ + *B. typhosus*.....	3	48

It would thus appear that this method can be employed for the purpose of differentiating bacteria. Bacteria so closely related as the typhoid-paratyphoid roup can be separated, so that there is every prospect that this method may

prove of considerable value in bacteriological investigations. Further experiments are now being made with other organisms which up to the present time have not been proved to be absolutely distinct.

Numerous experiments were made on the resistance of these endotoxins to heat, and every result shows that they are thermostable even when exposed to a temperature of 100° C. for 30 minutes, and are still able to exert their specific action, as is shown by the following experiment:—

	No. of bacilli in 50 cells.	No. of non-phagocytic cells.
A. Serum saline + leucocytes + *B. coli*	233	0
B. Serum Danysz extract + leucocytes + *B. coli*	59	15
C. Serum coli extract + leucocytes + *B. coli*	4	46
D. Serum Danysz extract* + leucocytes + *B. coli*	59	16
E. Serum coli extract* + leucocytes + *B. coli*	6	44

* This extract was exposed to a temperature of 100° C. for 30 minutes.

On the Determination of the Chief Correlations between Collaterals in the Case of a Simple Mendelian Population Mating at Random.

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(Communicated by Prof. Karl Pearson, F.R.S. Received June 3,—Read June 30, 1910.)

(1) In a paper communicated to the Royal Society in April, 1909, Prof. Pearson obtained the gametic correlations between the offspring and the ancestry in each grade in a simple Mendelian population mating at random. By a "simple Mendelian population" for a given character, he understood one which started with any definite ratio of dominant individuals (AA) to recedents (*aa*). These mating at random give rise to a population which may be written in the form

$$p^2(\mathrm{AA}) + 2pq(\mathrm{A}a) + q^2(aa),$$

and of which, without selection, the proportions of dominants, recedents, and hybrids are known to remain constant, with continued random mating, during successive generations. Prof. Pearson found that, both in the case of gametic and somatic characters, the ancestral correlations diminished in