The quantities of homologous protein sufficient to neutralise the precipitin in varying amounts of the same antiserum show some relationship to the amount of antiserum. The quantities of homologous protein augment with an increase in the amounts of antiserum, but whether the quantities of homologous protein are directly proportional to the amounts of antiserum is not ascertainable from the present data. The weights of the precipitates formed when increasing amounts of antiserum interact with a constant quantity of homologous protein insufficient to neutralise the whole of the precipitin in the antiserum augment with the amounts of antiserum, but the weights of the precipitates from each unit of antiserum diminish as the amounts of antiserum increase (Table III, tubes Nos. 10, 11, 12, and 13).

The weights of the precipitates from 1 c.c. of different antisera are of value in showing the order of magnitude of the weights of precipitin taking part in precipitin interactions.

In conclusion, I beg to express my indebtedness to Prof. Anderson Stuart, in whose laboratory this research was carried out.

The Influence of Bacterial Endotoxins on Phagocytosis.

(Preliminary Report.)

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These investigations were undertaken for the purpose of determining the effect of endotoxic substances on phagocytosis, as tested *in vitro*. Experiments were made to determine whether these substances, when added to a phagocytic mixture, would cause an increase or a decrease in the phagocytic activity; whether such action would be general or specific; whether the action would be affected by subjecting the endotoxins to varying degrees of temperature, and whether the toxins would act directly on the bacteria, the serum, or the leucocytes.

The present communication is intended only for the purpose of introducing our preliminary results, which are derived from a considerable amount of experimental enquiry. The explanation of the mode by which the effects to be described are produced is now under investigation.

Technique.—The organisms used in these experiments were as follows:—*B. Typhosus, B. Paratyphosus, B. Achard, B. Danysz, B. Coli* (several strains), *B. Friedlander, B. Proteus, B. Prodigiosus, B. Pyocyaneus, Micrococcus Aureus.* These were cultivated on agar at 37° C., and on gelatin at 22° C., for 24 to 72 hours. In one instance certain of the bacteria referred to above were obtained from broth flasks, which had been incubated for three weeks at 37° C. The bacterial extracts were made originally from cultures grown on the surface of the agar, but, for reasons which will be referred to subsequently, this medium was abandoned for gelatin.

To a sufficient growth of the organisms on plate cultivations a measured quantity of sterile salt solution was added, the entire growth was carefully removed, and the thick suspension of bacteria in saline transferred to a sterile agate mortar. The organisms were then ground up with considerable force in the presence of sterile powdered glass, or, in our later experiments, of sand. The bacterial extract was then transferred to sterile tubes, centrifugalised at high speed, and the supernatant fluid pipetted off into fresh tubes; this process was repeated until an extract was obtained free from bacteria. It was noted that the extracts, and more particularly those obtained from *B. Pyocyaneus* and *B. Proteus,* were often turbid, and that the turbidity was increased when the extracts were subjected to high temperatures. It was undesirable in the case of certain bacteria, namely, *B. Typhosus, B. Achard* and *B. Paratyphosus,* to obtain the extracts from the living organisms; in these instances the cultures were first killed by exposure to heat in the usual way, but similar results were obtained whether the extract was prepared from living bacteria or from those killed by heat. In a few instances the bacterial suspension was ground up after being frozen in an agate mortar surrounded by solid CO_2. The phagocytic mixture consisted of washed human leucocytes, pooled normal sera and 24 hour old suspensions in saline of living, never of heated, bacteria. The whole was incubated for 15 minutes at 37° C. In every case fifty leucocytes were counted with the number of bacteria engulfed, and the ratio of phagocytic to non-phagocytic cells noted.

It is convenient to mention here that extracts of organisms cultivated on agar were abandoned; because, in the majority of instances, the agar medium itself was found to directly inhibit phagocytosis. We employed agar media standardised to +1·5, +1·0, and +0·5 made in our laboratories, and also that derived from other institutions, but similar results were obtained in all cases; when gelatin was substituted for agar the results were constant, and free from error.

An essential point in the technique, and one upon which the most satisfactory results depend, is to procure a bacterial extract as concentrated as

possible. In our earlier experiments one part of bacterial extract was mixed with two parts of normal serum and either incubated for one hour at 37° C., and then one volume of the mixture added to equal volumes of leucocytes and bacteria, or the extract was added after 15 minutes incubation. It was found, however, that the best results were obtained by incubating for one hour at 37° C. equal volumes of serum and bacterial extract, and then mixing equal volumes of this with the leucocytes and bacteria. Incubation for 15 minutes failed to produce the striking effects shown when longer incubation was employed.

The Action of the Endotoxic Substance on the Leucocytes.

Owing to the limited number of experiments performed, this part of our investigation is incomplete. It would appear that if the endotoxin acts directly on the leucocytes at all, they are capable of complete recovery during the process of washing. Our experiments fail to indicate any such direct action on the leucocytes themselves.

The Action of the Endotoxic Substance on the Serum.

Equal parts of serum and bacterial extract were mixed together and incubated for one hour at 37° C. One volume of this mixture was then added to equal volumes of washed leucocytes and the bacterial suspension. The experiments were completed in the usual way. In every instance equal volumes of serum and normal saline were mixed and incubated for the same time, and used as a control.

The following experiments show the direct action of the endotoxin and serum mixture.

Experiment 1.*

A. Serum saline mixture + leucocytes + *B. pyocyaneus*—
50 cells contained 262 bacilli; non-phagocytic cells, 0.

B. Serum pyocyaneus extract + leucocytes + *B. pyocyaneus*—
50 cells contained 41 bacilli; non-phagocytic cells, 30.

Experiment 2.

A. Serum saline + leucocytes + *B. pyocyaneus*—
50 cells contained 390 bacilli; non-phagocytic cells, 0.

B. Serum pyocyaneus extract + leucocytes + *B. pyocyaneus*—
50 cells contained 12 bacilli; non-phagocytic cells, 43.

* In this and the following experiments by "serum saline," "serum pyocyaneus extract," etc., is meant one volume of a mixture of equal parts of serum and salt solution, or of serum and the bacterial extract in question, after one hour's incubation at 37° C.

Experiment 3.

A. Serum saline + leucocytes + *B. proteus*—
50 cells contained 474 bacilli; non-phagocytic cells, 0.

B. Serum proteus extract + leucocytes + *B. proteus*—
50 cells contained 33 bacilli; non-phagocytic cells, 24.

The following experiments demonstrate the completely specific action of the endotoxin:—

	No. of bacteria in 50 cells.	No. of non-phagocytic cells.
Experiment 1.		
A. Serum saline + leucocytes + *B. paratyphosus B.*	192	0
„ + „ + *B. Danysz*	62	16
B. Serum *paratyphosus B.* extract + leucocytes + *B. paratyphosus B.*	15	39
„ „ „ + leucocytes + *B. Danysz*	89	13
Experiment 2.		
A. Serum saline + leucocytes + *B. Achard*	249	1
„ + „ + *B. Danysz*	268	1
B. Serum *B. Danysz* extract + leucocytes + *B. Achard*	102	11
„ „ „ + „ + *B. Danysz*	25	33
C. Serum *B. Achard* extract + leucocytes + *B. Achard*	10	42
„ „ „ + „ + *B. Danysz*	123	11
D. Serum *B. coli* extract + leucocytes + *B. Achard*	156	2
„ „ „ + „ + *B. Danysz*	183	3
Experiment 3.		
A. Serum saline + leucocytes + *B. pyocyaneus*	234	0
„ + „ + *M. aureus*	99	20
B. Serum *B. pyocyaneus* extract + leucocytes + *B. pyocyaneus*	7	44
„ „ + „ + *M. aureus*	56	24
„ „ + „ + *B. pyocyaneus*	equal 7	44
„ „ + „ + *M. aureus*	vols. 36	36
C. Serum *M. aureus* extract + leucocytes + *B. pyocyaneus*	236	4
„ „ + „ + *M. aureus*	36	38
„ „ + „ + *B. pyocyaneus*	167	6
„ „ + „ + *M. aureus*	53	28

In the third experiment of this series, the specific nature of the endotoxic action is clearly shown when the leucocytes and equal volumes of cocci and bacilli are mixed together.

We may remark that the degree of phagocytosis in all our experiments is estimated by the number of organisms engulfed in a given number of cells, but

the ratio of active and non-active phagocytes is at least of equal importance. In these observations attention has been paid to the percentage of non-phagocytic cells, the importance of which has been previously insisted upon by many workers on phagocytosis.

When the reduction of phagocytosis by endotoxins had been proved,* investigations were undertaken for the purpose of ascertaining whether these toxic substances were resistant to heat. It was found that they were unaffected by exposure to a temperature of 60° C. for periods varying from 15 minutes to three hours, and that the results obtained with the heated substance were identical with those obtained with the unheated, as may be seen from the following experiment.

	No. of bacteria in 50 cells.	No. of non-phagocytic cells.
A. Serum saline mixture + leucocytes + *M. aureus*	182	9
„ „ + „ + *B. typhosus*	198	3
B. Serum *B. typhosus* extract + leucocytes + *M. aureus*	26	43
„ „ + „ + *B. typhosus*	17	38
C. Serum *B. typhosus* extract* + leucocytes + *M. aureus*.....	46	33
„ „ + „ + *B. typhosus*	15	37

* This extract has been exposed to a temperature of 60° C. for three hours.

The Effect of Diluting the Endotoxin.

The effect of diluting an extract before mixing it with the serum allowed a return to the same degree of phagocytosis as occurred in the serum saline mixture, *i.e.*, the control. Several notable exceptions were recorded, in which the diluted toxic substance appeared to play the part of a "stimulin." In such instances the degree of phagocytosis was far greater than in the control experiments. The stimulating action of the diluted toxin appeared to be specific. This may be briefly instanced by the following examples:—

* Prof. Hewlett very kindly suggested to us that we should ascertain whether similar results could be obtained by substituting *washed* bacteria for the preparation of the endotoxin, so as to eliminate the possibility that our results might be dependent upon an extracellular substance adhering to the unwashed organisms. Experiments were performed for this purpose and it would seem that washed bacteria can be employed for the preparation of the bacterial extract, although the results are not quite so striking as in the case of the unwashed. This fact could be explained possibly by the loss of bacteria in the washing, and, therefore, the small quantity of organisms left for grinding up.

	No. of bacteria in 50 cells.	No. of non-phagocytic cells.
Experiment 1.		
A. Serum saline + normal leucocytes + *B. paratyphosus B.* ...	230	2
B. Serum bacterial extract from *B. paratyphosus B.* + normal leucocytes + *B. paratyphosus B.*	32	23
C. Serum bacterial extract from *B. paratyphosus B.* diluted 1/10 + normal leucocytes + *B. paratyphosus B.*	492	0
Experiment 2.		
A. Serum saline + normal leucocytes + *B. prodigiosus*.....	117	5
„ + *B. typhosus*	181	9
B. Serum bacterial extract from *B. typhosus* diluted 1/10 + normal leucocytes + *B. prodigiosus*	153	4
Serum bacterial extract from *B. typhosus* diluted 1/10 + normal leucocytes + *B. typhosus*	372	0

A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Regular Periodical Increase of the Parasites Disclosed.

By Major Ronald Ross, F.R.S., and David Thomson, M.B., Ch.B., D.P.H.

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Prefatory Note by R. Ross.—For a long time it has appeared to me that much light might be thrown on infectious diseases, immunity and treatment, by more exact enumeration of the infecting organisms, and that we might even be able ultimately to apply mathematical reasoning to the study of these subjects. In 1903* I elaborated a method of blood examination, called the *thick-film process*, which enables us to detect small organisms in the blood about twenty times more easily than in ordinary preparations; but for the lack of the necessary assistance I was long unable to apply the method to the laborious enumeration of such organisms. Recently, however, the Advisory Committee for the Tropical Diseases' Research Fund has placed considerable funds at the disposal of the Liverpool School of Tropical Medicine for the study of cases in the tropical ward of the Royal Southern

* 'Lancet,' January 10, 1903, and Thompson Yates Reports, vol. 5, Part I, 1903.