Precision, capacity for truly quantitative measurements, uniformity of adjustment and rapid working, are among the most urgent desiderata. With the new instrument former work will be confirmed and rendered quantitative, while it is hoped to deepen and extend it in such manner as to lay sure the foundations of this new branch of practical science.

An Experimental Investigation into the Rôle of the Blood Fluids in the Intracellular Digestion of Certain Bacteria and Red Blood Corpuscles.

By S. R. Douglas, M.R.C.S., L.R.C.P. (Lond.), Captain I.M.S. (Retired), 1st Assistant, Bacteriological Department, Medical Research Committee, National Insurance Act.

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(From the Inoculation Department, St. Mary's Hospital, Paddington, W.)

Any observer who may have carried out a number of experiments on phagocytosis *in vitro*, especially when some members of the *coli* group, or the gonococcus, have been the microbes under observation, cannot fail to have been struck by the marked intracellular digestion which is seen in the microscopical preparations made in the course of these experiments.

This intracellular digestion is evidenced by many of the organisms ingested by the leucocytes appearing as ill-stained, swollen shadows lying in vacuoles.

Further, several independent workers in this laboratory, when working out the opsonic index in the case of glanders, noticed that those glanders bacilli which had been ingested by the leucocytes after being acted on by the normal human serum showed from their appearance in the opsonic films many more signs of digestion than those which had been acted on by the patient's serum, and this was the case quite independently of the value of the opsonic index of the patient's serum, as is shown by the fact that on occasions when this was especially noted the opsonic indices were 2·6, 2·7, 1·7, 2·3, and 0·96 respectively. Another observation bearing on this subject, and one which many workers may have noticed, is that the gonococci seen in the leucocytes contained in specimens of gonorrhœal pus are sharp cut, taking the stain evenly and deeply, whereas in specimens made for the determination of

opsonic indices to this microbe, however perfect the bacterial emulsion used may be, many of the organisms seen inside the leucocytes are so much digested as to make the counting of the individual cocci, necessary to obtain the average per leucocyte, a more than usually irksome task.

In view of the fact that there is present in every serum a very marked antidigestive property, namely, the antitryptic power, it would appear almost paradoxical that the serum might play a favourable *rôle* in a digestive process, yet on consideration it is quite conceivable that in the case of bacteria or red blood cells the serum might, by some action short of bacteriolysis or hæmolysis, render such bodies more permeable by the digestive fluids, and in this way favour their digestion.

Rosenow was the first to draw attention to this question, for in working with the pneumococcus he found that the amount of digestion which took place after these organisms had been ingested by the leucocytes varied considerably in different bloods, and he came to the conclusion that this variation was due to a property of the serum which acted on the leucocytes—not on the organisms—stimulating them to increased digestive efforts.

The experiments here detailed have forced upon me the conclusion that there is a property of the blood fluids independent of the opsonic power, which acts directly on the micro-organisms, or, as the case may be, on the red blood cells, rendering them more easily digested by the leucocytic ferments.

Experiments Made to Ascertain whether Red-Blood Cells or Bacteria which had been Acted on by Serum were capable of being Digested by Solutions of Trypsin or Leucoprotease.

Preliminary experiments having shown, firstly, that the author's serum had no hæmolytic power in regard to washed ox red blood corpuscles; secondly, that when washed ox red blood corpuscles were mixed either with his defibrinated blood, or with a mixture of his washed corpuscles and serum, large numbers of the ox red blood corpuscles were ingested by the leucocytes, and in them rapidly digested; thirdly, that even strong trypsin solutions showed no digestive action on washed ox red blood corpuscles, the following experiment was made:—

After washing ox red blood corpuscles free from all serum by repeated centrifuging with normal saline solution, a 10-per-cent. suspension was made. A series of tubes were now filled in with the following mixtures:—

Tube I.—One volume of 10-per-cent. suspension of ox red blood corpuscles and 2 volumes of fresh serum (S. R. D.'s).

Tube II.—One volume of 10-per-cent. suspension of ox red blood cor-

puscles and 2 volumes of serum (S. R. D.'s), which had been heated to 60° C. for 18 minutes.

Tube III.—One volume of 10-per-cent. suspension of ox red blood corpuscles and 2 volumes of normal salt solution.

These tubes were then incubated at 37° C. for one hour, after which, by repeated centrifuging and washing in normal saline solution, all traces of serum were removed. The red blood corpuscles thus obtained were then suspended in a volume of salt solution equal to the original volume of the suspension of ox red blood corpuscles, and an equal volume of a 1 in 10 trypsin solution was added. The tubes were again placed in the incubator at 37° C. for three hours, and after that period were put in the ice chest, remaining there overnight.

On examination the next morning, Tube I, that is the tube in which the ox red blood corpuscles had been acted on by the unheated serum, showed marked digestive changes, evidenced by hæmolysis and change of colour of the liberated hæmoglobin, whereas in the case of Tubes II and III, in which the ox red blood corpuscles had been brought in contact with heated serum or with normal saline solution before the addition of the trypsin, no digestion had taken place.

The conclusion that is drawn from this experiment, which was repeated on several occasions with identical results, is that the unheated serum acts in some way on the red blood corpuscles, so as to render them susceptible to digestion by trypsin.

Further experiments, with exactly similar results, were made, in which a leucoprotease solution was substituted for the trypsin solution. It was also found that normal rabbit's serum, although without any hæmolytic action on human red blood corpuscles, had the power of, in some way, acting on these cells, rendering them susceptible to digestion by solutions of trypsin.

However, human red blood corpuscles, which had been brought in contact with rabbit's serum which had been heated to 60° C. for a few minutes, were quite unaffected by such trypsin solutions.

Substituting bacteria for red blood corpuscles, a series of experiments showed that, in the case of a strain of *B. Friedländer* and some other coliform organisms, a very distinct digestion, evidenced by the loss of opacity of the emulsion, was brought about when such organisms had been previously acted on by unheated serum and afterwards treated with solutions of trypsin or leucoprotease, whereas when the organisms had been treated with serum heated to 60° C., or simply suspended in normal salt solution, both trypsin and leucoprotease solutions were quite inert.

These experiments show conclusively that the blood fluids modify the

red blood cells, or, as the case may be, the bacteria, in such a manner that they undergo digestion when brought into contact with solutions of trypsin or leucoprotease, solutions which have been found to be quite inert on suspensions of red blood cells or bacteria which had been previously acted on by heated serum or simply suspended in normal saline solution.

It is proposed to call this property of the serum the "protryptic" property of the serum, indicating that the serum by this action prepares the organisms or red blood cells for digestion by the leucocytic digestive fluids.

Experiments made to Ascertain whether only those Bacteria which had been Phagocytosed in the Presence of Unheated Serum were Liable to Undergo Intra-leucocytic Digestion.

Having found that plague bacilli are taken up by the leucocytes in considerable numbers, even in the presence of heated serum, an emulsion in normal saline solution of *B. pestis* was made from a 24-hour-old agar culture.

Human blood corpuscles (S. R. D.'s) were washed free from serum by centrifuging them in several changes of salt solution, and two samples of serum (S. R. D.'s) were obtained, one of which was heated to 60° C. for 10 minutes.

Two capillary tubes, such as are used in ordinary opsonic estimations, were now filled in with the following mixtures:—

Tube I contained:—

2 volumes of the washed corpuscles.
2 volumes of the unheated serum.
1 volume of the emulsion of *B. pestis*.

Tube II contained:—

2 volumes of the washed corpuscles.
2 volumes of the serum heated to 60° C. for 10 minutes.
1 volume of the emulsion of *B. pestis*.

These tubes were then incubated at 37° C. for four hours, after which films were made of samples of their contents, fixed in a saturated solution of corrosive sublimate and stained with carbol-thionin.

On examination, the microscopical preparations made from both tubes showed abundant phagocytosed bacilli, but whereas in the case of those made from Tube II, in which heated serum had been used, the bacilli lying in the leucocytes were deeply stained and perfectly sharp cut in appearance, in those made from Tube I, in which unheated serum had been used, the bacilli taken up by the leucocytes were almost completely digested, appearing as swollen, ill-staining shadows, frequently lying in well marked vacuoles.

This experiment was repeated again and again, sometimes with slight modifications, such as allowing the serum to act on the bacteria for varying periods before the washed corpuscles were added, but the result was always the same, namely, intra-leucocytic digestion could only be demonstrated when the organisms had been acted on by fresh unheated serum.

These experiments, although they furnished data showing conclusively that intra-leucocytic digestion only took place in the presence of unheated serum, were unsatisfactory, in that they failed to give any idea as to the proportion of organisms which were digested after being taken up by the leucocytes. The following experiments were therefore undertaken with the view of elucidating this point.

Phagocytic mixtures consisting of, on the one hand, washed corpuscles, unheated serum, and an emulsion of plague bacilli, and, on the other hand, of washed corpuscles, heated serum, and an emulsion of plague bacilli, were incubated at 37° C. in a water-bath.

At varying intervals samples of these mixtures were withdrawn and microscopical preparations were made and the number of bacilli contained in 100 leucocytes was ascertained.

In the case of the phagocytic mixture which contained the unheated serum it was found that the number of bacilli that could be recognised in the leucocytes became smaller and smaller the longer the tubes were incubated, at any rate up to a period of four hours, and from these figures it was possible to make a rough estimation of the number of organisms that had been digested.

In the case of the mixture which contained the heated serum the result was completely different, since each successive sample showed that the leucocytes contained larger and larger numbers of bacteria and these appeared normal as regards both their shape and staining reaction.

The details of one such experiment are here given.

A very thick emulsion of plague bacilli, from a 24-hour-old agar culture, was made in normal saline to which 1 per cent. of formalin had been added. The emulsion was kept at room temperature for about one hour to allow the formalin to act on the bacilli.

This procedure, which killed the organisms, was necessary, as when similar experiments were made with emulsions of living plague bacilli it was found that after incubation at 37° C. for some hours a proportion of the bacilli which had been ingested by the leucocytes were capable of multiplying, and in consequence of this the results obtained were irregular and fallacious.

This thick emulsion was now diluted 100-fold with normal saline, so that the concentration of formalin in the final phagocytic mixture given below was

reduced to 1 in 6000, a strength which has little or no inhibitory effect on phagocytosis.

Two small test-tubes were now filled in with the following mixtures:—

Tube I. 250 c.mm. of washed corpuscles.
250 c.mm. of unheated serum.
100 c.mm. of the emulsion of plague bacilli.

Tube II. 250 c.mm. of washed corpuscles.
250 c.mm. of serum heated to 60° C. for 20 minutes.
100 c.mm. of the emulsion of plague bacilli.

Both tubes were incubated in a water bath at 37° C. and after stated intervals samples from each were removed with a capillary pipette. From these, films were made which were fixed with a saturated solution of mercuric chloride and stained with carbol thionin.

The number of bacilli contained in 100 leucocytes was now estimated.

This was by no means an easy task in the case of some of the films made from the samples taken from tube I, in which owing to the presence of the unheated serum the bacilli were undergoing intraleucocytic digestion.

The numbers given below include not only the organisms which, because of their perfect shape and staining, were deemed unaffected by the leucocytic digestive fluids, but also many which showed signs of commencing digestion.

Time of incubation.	Number of bacilli in 100 leucocytes in samples from Tube I, containing unheated serum.	Number of bacilli in 100 leucocytes in samples from Tube II, containing heated serum.
15 minutes	330	80
30 „	280	140
1 hour	210	210
2 hours	95	280
3 „	57	250
4 „	27	300

On examining the figures thus obtained, assuming that no more bacilli were ingested by the leucocytes after the first 15 minutes' incubation, the only conclusion that can be drawn is that 90 per cent. of those organisms which were ingested in the first 15 minutes have been so completely digested during the succeeding $3\frac{3}{4}$ hours that they no longer can be recognised as bacteria.

But most probably what does happen is that the leucocytes continue to take up microbes to some extent during the whole period of incubation, so that in reality even a greater percentage than 90 per cent. of those microbes which were ingested by the leucocytes in the first 15 minutes have been

completely digested during the succeeding $3\frac{3}{4}$ hours the phagocytic mixtures were incubated.

When the figures given by the films made from the samples taken from tube 2, which contained heated serum, are examined, it is found that, instead of the number of bacilli per 100 leucocytes diminishing, there is a steady increase until, after the incubation of this tube had continued for four hours, the number of organisms found to be ingested in 100 leucocytes approximated to the number ingested through the action of the unheated serum in 15 minutes.

Further, even after incubation had continued for four hours the bacilli lying in the leucocytes showed practically no signs of digestion.

Summary and Conclusions.

These experiments showed that, as regards the particular bacteria and red blood corpuscles, and also as regards the blood fluids used in carrying them out :—

1. The blood fluids have the property of influencing the digestion of such bodies as red blood corpuscles and bacteria taken up by the leucocytes.

2. This action of the blood fluids is quite independent of the opsonic action, this being shown by the fact that intracellular digestion may be more marked as the result of the action of a serum of lower opsonic power than of a serum of much higher opsonic power.

In these two conclusions the author is in complete agreement with the conclusions drawn by Rosenow.

3. The power of the blood fluids to prepare such bodies as red blood cells or bacteria for digestion by solutions such as trypsin and leucoprotease, or by the digestive fluids which are secreted after such bodies are ingested by the leucocytes, is not, as stated by Rosenow, due to stimulation of, or an action on the leucocytes, but is due to a direct action on the bacteria, or, as the case may be, the red blood corpuscles. This is demonstrated by those experiments in which the red blood corpuscles or bacteria, after being brought in contact with fresh serum, which was subsequently removed, were found to be digested by solutions of trypsin or leucoprotease, solutions which had been previously shown to be quite without action.

4. Heating the serum to 60° C. destroys the property of the serum to prepare such bodies for digestion, at any rate in the case of normal serum.

5. It is proposed to name this property of the blood fluids the " protryptic " power of the serum, seeing that it prepares such bodies as red blood corpuscles and bacteria for solution by the digestive fluids secreted by the leucocytes or by solutions of trypsin.