While the observations in this paper have been confined to tuberculous infections, it may be surmised that this diagnostic blood reaction to an injection of a dead bacillary suspension might be adopted with corresponding success in other infections; and such, in fact, has been our experience. We leave this point to be dealt with on another occasion.

The opsonic estimations were carried out in the University Surgical Laboratory, and we gladly take this opportunity of thanking Professor Chiene for every facility granted to us there.

The patients were for the most part attending the Surgical Out-Patient Department of the Infirmary. To the surgeons in charge of that department, and to other members of the staff, we gratefully acknowledge the material placed at our disposal.

PHAGOCYTOSIS OF ERYTHROCYTES, AND THE QUESTION OF OPSONIN IN PAROXYSMAL HÆMOGLOBINURIA.

By J. EASON, M.D., F.R.C.P.Ed., *Assistant Physician, Leith Hospital.*

AT the present time a controversy of considerable interest is proceeding, the point at issue being to determine the identity of the substance in the blood which exercises the opsonic function. More precisely, the point to be determined is whether the so-called opsonin of Wright and Douglas possesses characters which prove it to be a substance which differs from the amboceptor or immune body; that is to say, does it lose its power of opsonising on being heated for thirty minutes at 55° C., and also on being kept *in vitro* for some days; or does it, like the amboceptor, retain its power after such treatment?

Dean's paper in the *Proceedings of the Royal Society of London* goes far to prove that the term "opsonin" applies to the substance which is already known by many names, among which may be mentioned *amboceptor, fixateur, immune body, intermediary body, substance sensibilisatrice*, etc.

At the same meeting of the Royal Society, Wakelin Barratt described a series of experiments which led him to conclude that the substance which causes phagocytosis of *erythrocytes* in a hæmolytic serum is thermo-labile. Consequently he thought the substance "is a special constituent, which is a member of the group of opsonins first described by Wright and Douglas in respect of bacteria." To this substance Barratt applies the name *hæmopsonin*.

Now there is, of course, nothing new in the idea that an immune bacteriolytic or hæmolytic serum induces increased phagocytosis of the cell or micro-organism to which immunity exists.

Such phagocytosis is the basis of the Metchnikovian theory of immunity. Moreover, the extraordinary eagerness of the phagocytic action towards bacteria and erythrocytes in the presence of an immune serum has been demonstrated by such investigators as Savtchenko, Ruziczka, Levaditi, and Gruber, by experimental work on animals.

In a former paper I have already quoted the works of these authors, and Savtchenko's work occupies an important place in the recent literature of immunity. The above-mentioned investigators satisfied themselves, by careful and ingenious work, that the substance inducing the phagocytosis was the immune body. This work antedated that of Wright and Douglas, by whom the technique has been adapted for clinical purposes.

The only disease occurring in the human subject on which Wakelin Barratt's views on hæmopsonins appear to have any practical bearing at present, is paroxysmal hæmoglobinuria. In this disease it has been shown by Donath and Landsteiner, and myself independently, that the hæmolysis depends on the activity of an intermediary body or amboceptor, whose activity is in abeyance if the blood is maintained (*in vitro*) at the normal temperature. Under such circumstances no hæmolysis occurs. But if a tubeful of blood is allowed to cool in ice water after being mixed with 0·25 per cent. pot. oxalate in 0·85 per cent. saline solution to prevent coagulation, and is thereafter placed in an incubator at 37° C. for a short time (three hours), hæmolysis occurs, while control tubes of normal blood, under similar conditions of temperature, show no hæmolysis.

If microscopic preparations be made from the tube containing the pathological blood after it has been thirty minutes in ice water, and a similar period in the incubator at 37° C., it will be found that a very active solution of the erythrocytes by the phagocytes is proceeding.

It was this observation, which I have frequently made since 1903, that first enabled me to conclude that we had to do with the presence of a hæmolytic amboceptor in the blood of paroxysmal hæmoglobinuria. Such a conclusion appeared justifiable in view of the evidence which I have above quoted from the investigations of Savtchenko, Gruber, Ruziczka, and others, that such phagocytosis in experiments on animals was the result of the union of amboceptor and erythrocyte.

I have previously described the nature of this phagocytic process which is, at least, *as greatly participated in by the polymorpho-nuclears as by the mono-nuclears*. By reference to my previous paper in this Journal, and to the investigations of Gruber and Ruziczka, it will be seen that this process is a phagocytic one, but that the erythrocyte is dissolved at its point of contact with the phagocyte, and it is therefore partly an extra-cellular process, although the hæmoglobin becomes absorbed. Large vacuoles about

the size of a red corpuscle may be seen sometimes in the polymorphs. These may be blood corpuscle shadows, but of that I am not yet convinced. Phagolysis sometimes appears to be proceeding as rapidly as hæmolysis, and the erythrocytes appear to undergo very rapid solution in the medium (complement?) thus set free from the leucocytes. This process may also be observed if the inactivated serum of a case of paroxysmal hæmoglobinuria is mixed with washed corpuscles and leucocytes from a normal individual, and is submitted to the condition of temperature already described. This form of phagocytosis would therefore not appear to be dependent on the action of a thermo-labile substance or normal opsonin, but of a thermo-stable substance which may be either the hæmolytic amboceptor or a body corresponding to the thermo-stable opsonising substance which, according to Muir and Martin, is artificially produced by immunising an animal.

These results are therefore not in agreement with the observations of Wakelin Barratt, but with those of Dean on the phagocytosis of bacteria. Again, my own observations in paroxysmal hæmoglobinuria, and those of Gruber and the recent work of Keith, agree in finding the polymorphs as very active phagocytes for the erythrocytes. Barratt maintains the older view, that the mono-nuclears are chiefly concerned in this process.

The observations which I have above described may be made clinically by employing the method originally used by Ehrlich to simulate the conditions of a paroxysm. An elastic ligature is put round a finger of the patient. The finger is then put in ice water for a short time (five to ten minutes), and is then put in warm water for a similar period of time. Blood is then obtained in the usual way for film-making, and stained.

Thus it appears that the phagocytosis is as much dependent on the previous chilling of the blood as is the extra-cellular lysis, another indication that the amboceptor is the cause of both processes.

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