THE PATHOLOGY OF PAROXYSMAL HÆMOGLOBINURIA (PRELIMINARY COMMUNICATION).[1]

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(Plate I.)

Since Dressler published the first satisfactory description of a case of paroxysmal albuminuria and chromaturia in 1854, the pathology of this disease—now known by the more appropriate name, paroxysmal hæmoglobinuria—has had devoted to it a very considerable amount of investigation, which, however, has not given results sufficing to explain its pathology. The literature of paroxysmal hæmoglobinuria is very copious, and the theories regarding its pathology have been so numerous and varied that it would now require considerable space to give a complete synopsis of them. Many of these theories are speculative opinions of little or no value.

The present research is indeed not intended to be a complete historical account of already published writings, although many of those which are of permanent value must necessarily be alluded to whenever consideration of the pathology of the disease is entered upon. My chief object is to describe certain experiments which I have been enabled to conduct by the methods of research introduced in quite recent years by Ehrlich and Morgenroth.

Since the publication of Chvostek's monograph a new epoch in medical science has begun with the work of Bordet and Ehrlich on immunity, and the initiation of the present research was due to the general knowledge I possessed of those famous investigations. It occurred to me that the clinical features of paroxysmal hæmoglobinuria were such that an investigation of the disease by the methods of Ehrlich was required; and the most careful consideration that I gave to all the circumstances of the disease encouraged me to form the opinion that the pathology of paroxysmal hæmoglobinuria would ultimately be explained by the information thus obtained.

I began in 1903 with the working theory that we have probably to deal with a hæmolysin which might or might not be composed of two active substances, amboceptor[2] and complement.[3]

The following possibilities occurred to me regarding the supposed blood toxin:—

It might be—(1) A toxin whose usual habitat was not the blood, but whose passage into the blood was determined by such factors as cold on the surface of the body, or muscular exertion. (2) A toxin whose development in the blood was promoted by

[1] Part of a thesis for the degree of M.D., and which was awarded a gold medal. Read before the Galenian Society, January 1904.

[2] Intermediary body, fixateur, etc. [3] Alexin, etc.

such factors. (3) A potential toxin which acted only under the influence of such factors. (4) A combination of two or more of these.

The theory that this disease is consequent on the presence of a toxic substance in the blood, was first suggested by Wiltshire in 1870, and in the time that has elapsed occasional opinions have been expressed in support of such a theory. Wiltshire's view was, that the "disintegrating agent" was a volatile organic acid, probably a fatty acid which was thrown back into the system by chilling of the skin, and thereby "autogenetic poisoning" occurred.

Ilgner considered it an infectious disease. His patient had malaria previously. Ehrlich's original view was that a proportion of the red cells were hypersensitive to the action of cold. He maintained that this was proved by ligaturing and immersing a finger in ice-cold water for a quarter of an hour and then in tepid water for a quarter of an hour. The serum was then hæmoglobinæmic. In a later publication he found he could not confirm this *in vivo* experiment by a somewhat similar one *in vitro*.

He then concluded that the blood vessel endothelium under the influence of cold, produces substances (ferment) which destroy the corpuscles.

Charpentier thought that under the influence of cold combined with malarial infection there was produced a toxin which could disintegrate the corpuscles directly or by indirect action through the vasomotor centre. His views were not supported by any experimental investigation.

Prior investigated the literature and conducted much work, to which reference will be made later. He formed the opinion that a toxic substance originates in the alimentary canal and passes into the blood circulation. Such a view has some resemblance to that of Hunter in the case of pernicious anæmia. Chvostek considers the formation of toxic material by the vessel endothelium at least improbable, because destruction of corpuscles takes place *in vitro* by shaking.[1] An abnormal property of the serum cannot be demonstrated.

Hayem in 1889 indicated that there was probably a toxæmic condition of the blood in this disease. He has recently given his views on this subject in his published lectures. The paroxysm is considered to be due to a toxæmia in individuals affected with a chronic alteration of the blood. Assuming this change in the blood, he considers that the paroxysm can then be determined by causes which increase the waste products circulating in the blood, such as cold and fatigue. He refers to the syphilitic origin of many of the cases, and believes that this accounts for the blood in these cases being rendered vulnerable, just as in paludism the blood is rendered vulnerable to quinine. In the absence of syphilis the corpuscles may be rendered vulnerable by other causes,

[1] *Loc. cit.*, p. 65.

such as gastritis of alcoholic origin which was present in one of his cases.

In short, his view is that cold or fatigue produce toxic substances which, while they do not cause the destruction of normal cells, are yet sufficient to destroy the more vulnerable cells of individuals predisposed by a certain toxæmic state of the blood. His chief reason for accepting a toxic origin is the presence of methæmoglobin in the urine, and he considers the corpuscle destruction takes place in the kidney, where alone the toxic substances would be present in sufficient concentration to produce this change.

Luzzatti and Sorgente think that the blood serum during the paroxysm has a hæmolytic action. The serum of the interval has no abnormal or strong hæmolytic action, but the serum of the stasis blood produces quick hæmolysis unless the part affected by stasis is kept warm. If the ligatured limb is kept warm, the serum separated from the blood is much clearer than if the unligatured limb is subjected to chilling before taking away blood. This experiment minimises the intrinsic importance attributed to the action of stasis by Chvostek.

Luzzatti and Sorgente, agreeing with Hayem, believe that serum which separates, coloured by hæmoglobin, has no further hæmolytic action. After the paroxysm the serum resumes its normal conserving power. Contrary to the view of Hayem, they believe that destruction takes place in the peripheral vessels and not in the internal organs as a rule. They do not deny that there may occasionally be cases of renal origin. Blood obtained from their patient in the interval gave up a clear serum—(*a*) After coagulation at the room temperature or on ice, (*b*) by centrifuging. But if the temperature was changed from that of ice to 37° C., a rose-red serum was sometimes obtained. To this they attached no importance, as by the same means a coloured serum was obtained from the blood of healthy children. After many experiments *in vivo* and *in vitro*, they concluded that cold produces certain changes within the organism which cannot be produced by this agent *in vitro*.

In regard to the toxic theory of paroxysmal hæmoglobinuria, the foregoing opinions are those which had already been published when an opportunity presented itself to me to study two cases of this disease which came under my observation at the out-patient department of Leith Hospital.

After full consideration of the chief features of the disease, and keeping in view certain facts and ideas regarding malaria and pernicious anæmia, I became eager to know if in the case of paroxysmal hæmoglobinuria we had to do with the action of a hæmolytic body, and if so, then to ascertain if this body produced its action in the same way as Ehrlich and Morgenroth have maintained for the immune sera, namely, by means of amboceptor and complement.

Technique.—This varied to an extent which will be defined as I proceed with the description of the various experiments. At first it was desirable to ascertain by the simplest methods if there were sufficient reasons for proceeding with a lengthy investigation, in which the more elaborate experimental technique of Ehrlich could be employed.

On that account the plan of using serum obtained from cantharides blisters appeared worthy of trial. There was the advantage, moreover, that a large amount of serum could thus be readily obtained. From a blister 2½ in. square, 10 c.c. of serum are usually available. Previous to the application of the cantharides plaster, the skin was carefully cleansed with lysol and water, then with methylated spirit, and finally with boiled water. Before opening the blister, its surface was again very carefully washed with boiled water. A Graefe knife and scissors were sterilised in boiling water, and a test tube was washed, dried, and plugged with wool, and finally kept in an oven for two hours at 160° C. A small puncture was then made in the most dependent part of the blister, and the serum was received directly into the tube, which was again closed with the sterile plug. I thus obtained in a very simple way a large amount of serum. In order to test the toxic properties of the serum on corpuscles of the same or another individual, the manipulation was again simple, and therefore conducive to asepticity. One c.c. of the serum was drawn up into a sterilised pipette, and was then received into a test tube already sterilised. A finger of the individual who was to supply the blood was carefully cleansed with lysol solution, and then with boiled water, and dried with wool. A surgical needle sterilised in boiling water was used to prick the finger, and three drops of blood were allowed to fall into the serum. The woollen plug, sterilised with the tube, was then replaced, and the serum and blood were gently shaken. In the earlier experiments the tubes were allowed to stand at the room temperature for twenty-four hours. It will be noted that the whole blood was used (not corpuscles which were separated and washed from the serum). Several very careful workers have likewise used whole blood, as for instance Polk, in a recent research on the heterolytic action of human serum on rabbits' blood. Polk considered his results in no way vitiated by doing so. This technique was also quite satisfactory for my preliminary experiments.

In July 1903, the patient, T. S., was in hospital, and had many paroxysms. He was certain to have a paroxysm if he remained up out of bed for an hour or two.

On 10th August, T. S. was allowed up for two hours. He began to shiver, and the characteristic symptoms of a paroxysm followed. A blister was applied to the abdomen, and the serum was obtained on the following day (serum A). Serum was also obtained from a man who had just recovered from acute rheumatism (serum B), and a girl suffering

from nephritis (serum C). (1) Blood was obtained from the man who gave serum B; (2) blood was obtained from resident physician; (3) blood was obtained from myself.

1 c.c. Serum A + Blood 1, three drops	}	Serum had a deep carmine colour.
1 „ „ A + „ 2, „		
1 „ „ A + „ 3, „		
1 „ „ B + „ 3, „		Serum remained very pale.
1 „ „ C + „ 3, „		Serum very slightly tinted.

The clot which formed in the tubes varied thus—

Blood mixed with serum C gave a "tough and heavy" clot.
„ „ „ B „ "dense and heavy" clot.
„ „ „ A „ "much less dense clot than in B and C.

Microscopical examination of the corpuscles after ninety minutes in the tube disclosed variation in the size of the cells acted on by serum A. There was no tendency to rouleaux formation. After twenty-four hours there was considerable variation in the size of the cells. The small cells were much crenated and deeply coloured. Other cells were large and pale.

With the microscope, the serum could also be observed to have a deeper colour. Cells acted on by serum B were grouped together after ninety minutes. Variation in size also present. After twenty-four hours, the cells, besides showing grouping, were almost without exception small, and their colour was uniformly deep. Not much crenation.

Serum A, therefore, appeared to have the property of injuriously acting on the red cells, and also that of modifying the fibrin formation. This serum could not accurately be called the serum of a paroxysm, as the blister was only applied five hours after symptoms began, and the serum was not obtained till the following day.

Similar experiments were done on 12th August, and again on 16th August, when there could be no doubt that it was *interval* serum. The former results were confirmed.

August 23.—In order that the serum of the *paroxysmal* period might be tested, I availed myself of the patient's predisposition to paroxysms if allowed out of bed.

Accordingly, a blister was applied at 5 A.M., and the patient was allowed out of bed at 10 A.M. At 2.45 P.M. he began to be shivery. At 3.15 P.M. he passed dark urine containing hæmoglobin. Meantime the blister had been forming, and was likewise evacuated at 3.15 P.M. The serum was golden in colour, and a spectroscopic examination showed the bands of oxy-hæmoglobin. There was, however, so little hæmoglobin present that the naked-eye appearance did not suggest its presence. There was absolutely no reddish tint. The following experiments were done:—

1.	1 c.c.	normal serum	+	normal blood,	three drops.	No hæmolysis.
2.	1 „	„	+	P.H.	„ of interval.	No hæmolysis.
3.	1 „	P.H.	+	normal	„ three drops.	Hæmolysis.
4.	1 „	P.H.	+	P.H.	„ of interval.	Great hæmolysis.

Evidently, the serum of the paroxysm had a similar action to that of the interval. On this occasion the mixtures were shaken up somewhat violently, and therefore the negative result in 2 speaks strongly against Chvostek's theory.

Chvostek observed that the serum became hæmoglobinæmic if the blood of his patient was put in a centrifuge which did not move smoothly, and the same occurred in the serum of blood obtained by wet cupping. These observations led him to conclude that the corpuscles were very susceptible to mechanical injury, and that hæmoglobinæmia resulted from this cause. My last experiments indicate that Chvostek's theory is at least not an entirely satisfactory one. The corpuscles in tube 2 withstood a considerable degree of violent shaking.

Donath has recently reported on the resisting power of the red cells in this disease after a careful investigation, and he has found that the corpuscles of paroxysmal hæmoglobinuria blood were always less resisting (in three cases) than those of normal blood. Full consideration of this aspect of the subject is meantime reserved. The results I have recorded, however, distinctly indicate that there is some other explanation of the hæmolysis than that of Chvostek. I hope to show that Donath's results are not inconsistent with my view. I am, moreover, convinced from the experiments on 10th, 12th, and 16th August that mechanical injury is by no means a necessary condition for the occurrence of hæmolysis.

The method of using blisters to test the serum of the paroxysmal period was very satisfactory, as blood serum obtained at this period is generally so much coloured that reliable results cannot be easily obtained.

The following microscopical examination was made :—

A drop of T. S.'s serum was placed on a glass slide (1) along with a drop of normal blood, and a cover-slip was then applied. A similar preparation (2) was made with the control serum, which in the last tube experiment was found to possess no hæmolytic action on normal blood. The two slides were then laid on a sheet of moistened blotting paper, which was placed under a bell jar for one hour. The following characteristics were then noted on microscopical examination :—

Slide 1. (1) No rouleaux formation.
,, (2) Variation in size of cells.
,, (3) Poikilocytosis fairly marked. The cells assuming a lenticular shape.
,, (4) There also appeared to be a change in the condition of the pigment, which now appeared pink.
,, (5) Practically no crenation.

Slide 2. (1) General formation of rouleaux.
,, (2) No variation in the size of cells.
,, (3) No poikilocytosis.
,, (4) No pink appearance of cells.
,, (5) Crenation distinct.

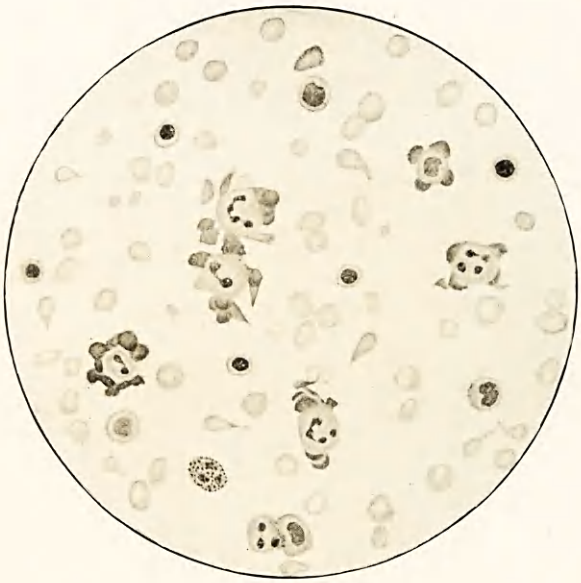

FIG. 1.

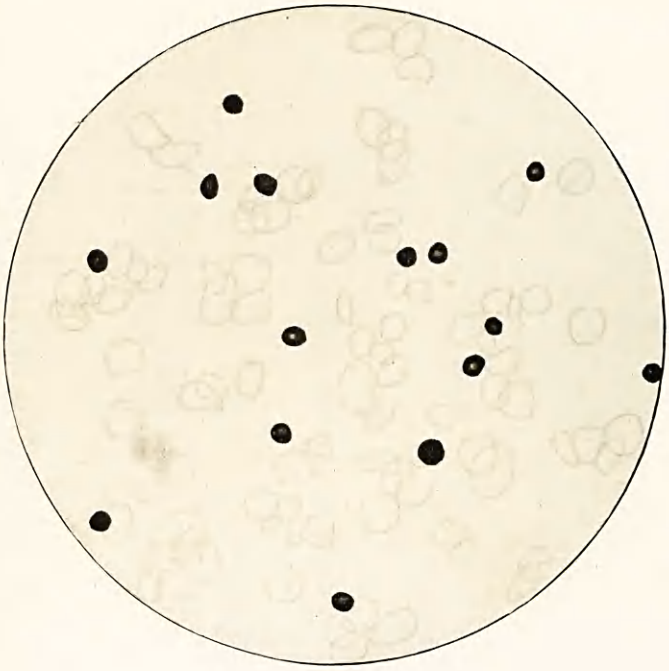

FIG. 2.

On 30th August (during a paroxysm), blister serum was again obtained, and its hæmolytic action on normal blood corpuscles (my own) was again noted. A careful microscopical examination of the cells was made after the tube had been allowed to stand for sixty minutes at the room temperature. Their condition is fairly well indicated in Plate I., Figs. 1 and 2. Already after sixty minutes many changes have become evident in the cells. In the size and colour of the red cells there is considerable variation, while some may be considered normal in these respects. There are many large cells which are very pale, and there are others which have become contracted, and possess a uniform supernormal colour. Changes in the condition of the hæmoglobin within the cells are also present. In numerous cells globules of dissolved hæmoglobin may be seen dotted throughout the cell. Some of these globules I have seen lying free in the serum, apparently so recently extruded from the cell that sufficient time had not elapsed in order to permit an intimate mixture of the hæmoglobin with the serum.

I wish to emphasise that the above description refers to the action of paroxysmal hæmoglobinuria serum on normal cells (isolysis) which were originally of uniform size.

I also made a most interesting observation which jointly concerns the leucocytes and erythrocytes. I attach considerable importance to the observation, for reasons which I will now submit.

Levaditi, by his investigations in the domain of immunity, was one of the foremost to draw attention to the extraordinary activity with which phagocytes attack red cells which have become loaded with intermediary body. He found that the red cells when in this condition are very eagerly absorbed by the phagocytes, in whose interior solution then occurs. This appears to be an extreme degree of the process already observed by Metchnikoff.

Savtchenko has made the same observation as Levaditi.

Gruber conducted many experiments to test this and other observations made by Levaditi. He entirely agrees with Levaditi in maintaining "that erythrocytes, which contain intermediary body, undergo phagocytosis much more quickly and in much greater number than do normal erythrocytes."

Ruziczka has also noted this extraordinary phagocytic attack on the red corpuscles in the presence of intermediary body.

Ruziczka immunised guinea-pigs to fowls' blood, and then, after immunisation, the fate of the blood corpuscles of later intraperitoneal injections was ascertained (after some time had elapsed), by obtaining some peritoneal contents by means of a capillary tube. He observed that the microphages and macrophages are able to destroy the red corpuscles without previously absorbing them, but by fixing themselves to the red cells, and gnawing and digesting them away bit by bit, apparently by the action of a digesting juice.

Those corpuscles which are thus nibbled away do not swell up (as when destroyed free in the serum by the complement), nor

do they lose their hæmoglobin, but retain it to the last, and indeed possess a more intense colour than the intact corpuscles. This process can be followed beautifully *in vitro*.

The above description is a literal translation from Gruber, who has completely satisfied himself of the accuracy of Ruziczka's observation.[1]

I have said that in the microscopical examination of normal human blood which had been exposed to the action of paroxysmal hæmoglobinuric serum, I had made an observation which concerned jointly the leucocytes and erythrocytes. It was this phenomenon described by Ruziczka and Gruber to which I referred, and the reason I deemed my observation of importance was that which I have just stated, namely, that Levaditi, Savtchenko, Gruber, and Ruziczka are agreed that such unusual eagerness of the phagocytes is the result of the activities of intermediary body on the red corpuscles.

The deeper colour possessed by those red cells which are attached to the leucocytes is shown in Plate I., Fig. 1. It may also be seen that those attached cells have not become enlarged.

In many cases also the red cells appear to be partly nibbled away. The significance which I attach to those changes which I have observed in the red and white cells is that we have probably to do with the activities of an intermediary body in the serum of individuals affected with paroxysmal hæmoglobinuria.

A series of experiments was thereafter done with the object of determining the effect of temperature on the process of hæmolysis, by paroxysmal hæmoglobinuric serum. The result of these experiments was that all preparations which were kept at the blood temperature showed no sign of hæmolysis, while, as before, those which were allowed to stand for some time at the room temperature showed distinct hæmolysis. Meantime, I reserve the detailed description of these experiments.

Control experiments.—Control blister serum experiments were also done in such diseases as rheumatism, epilepsy, tuberculosis, myxœdema, pleurisy, pneumonia, cancer, rheumatoid arthritis, etc., as well as with serum obtained from many apparently healthy individuals. In almost all cases no hæmolysis occurred, and never with serum from those having good health. In the very few instances in which hæmolysis occurred, it was *always less pronounced than that produced by paroxysmal hæmoglobinuric serum.* I hope to give further data at another time to indicate the diseases in which the serum may have hæmolytic properties.

Blood serum experiments with paroxysmal hæmoglobinuria serum gave similar results to those already indicated in the case of blister serum. The same conditions of temperature were required for hæmolysis.

[1] Ruziczka's work is published in the Bohemian language.

The following experiment may be quoted as being a further help to understanding the mode of action of the toxin :—

November 12.—Blood serum obtained from the patient, M. R., during interval. Under aseptic precautions, blood was obtained from a vein of the arm, by means of an exploring syringe. The blood was allowed to coagulate in the syringe, and when the serum had separated it was drawn up by means of a fine pipette. The serum was clear and yellow.

The serum was diluted with an equal part of 0·5 per cent. NaCl solution in order to have a workable quantity of fluid. One-half of this was inactivated by keeping it at 56° C. for an hour.

1. 0·75 c.c. active serum + 0·75 c.c. 5 per cent. mixture of normal washed red blood corpuscles in 0·5 per cent. NaCl solution. Distinct hæmolysis in three hours.
2. 0·75 c.c. inactivated serum + 0·75 c.c. 5 per cent. mixture of normal washed red blood corpuscles in 0·5 per cent. NaCl solution. No hæmolysis.

The tubes were exposed to the room temperature for three-quarters of an hour before being placed in the incubator at 37° C.

Hitherto I had rarely obtained hæmolysis as quickly and, in the course of three hours, never in such a pronounced degree. The negative result obtained in tube 2 was a corroboration of my supposition that the hæmolysis was effected by means of intermediary body and complement.

I have already indicated my reason for supposing that an intermediary body was present in the serum. This is a thermo-stabile substance, as Ehrlich and many subsequent writers have demonstrated. It remains uninjured by exposure for half an hour to a temperature of 56° C.

I expected, however, to be able to prevent hæmolysis by rendering inactive a second substance, namely, the complement or thermo-labile component of the hæmolysin. The result as shown by 2 agreed with my anticipation.

Although a slightly hypotonic salt solution was used in the above experiment, the positive result in (1) is not invalidated thereby, for the reason that hæmolysis of normal corpuscles, so prepared, has not occurred within three hours in my experience, nor was there hæmolysis in (2).

This concludes the brief outline of a series of experiments performed during 1903, and on consideration of the results which were obtained I was enabled to arrive at the following conclusions :—

1. A pathological substance is present in the blood serum and lymph of individuals affected with paroxysmal hæmoglobinuria.
2. This substance can dissolve (*in vitro*) the corpuscles of the affected individual (autolysis) and also those of normal individuals (isolysis), provided suitable conditions as to temperature exist.
3. Temperature conditions require to be further studied ; it is, however, apparent that a temperature much below that of the

body favours the action of the hæmolytic substance, while the normal body temperature retards or prevents it.

4. My observations regarding phagocyte activity during the hæmolysis (*in vitro*) correspond with those made by Levaditi, Savtchenko, Gruber, and Ruziczka, in their comparative work on immunity.

5. These authors consider that the excessive phagocytic action, observed by them, is significant of the antecedent union of intermediary body with the red corpuscles. "The Ruziczka phenomenon" is constantly observed during the autolysis and isolysis of red corpuscles produced by the serum of paroxysmal hæmoglobinuria cases. Therefore the probable explanation of the hæmolysis is the union of intermediary body and red corpuscles.

6. The intermediary body of paroxysmal hæmoglobinuria, however, cannot of itself produce solution of red corpuscles. For this, the presence of a thermo-labile substance complement is also required.

7. The changes observed in the red cells in course of solution correspond with those produced during hæmolysis by an immune serum as described by Gruber.

8. The serum of normal individuals does not cause hæmolysis. The serum of various individuals suffering from disease produced little and generally no hæmolysis.

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