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# PAROXYSMAL HEMOGLOBINURIA.*

## ACCOUNT OF TWO CASES.

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The first of the two patients, A, who furnish the basis of this report is a man 29 years old, who was born in Hungary, and served as a soldier in that country. During his military service he suffered much from chilblains during the winter, but he never had an attack of hemoglobinuria until December, 1902, eight months after coming to America.

During the summer of 1902 he lived in Saginaw, Mich., where he suffered much from mosquito bites.

There is absolutely no history of any infection or illness until December, 1902, when the patient first observed the attacks of hemoglobinuria, which were invariably associated with exposure to cold. We had no opportunity to observe any chilblains in this patient, but he says that when his feet or hands are thoroughly chilled they become very white, then blue and finally red, swollen and feverish. The swelling lasts several hours. According to the patient's account of himself, such an attack is always followed by a "black-water" attack.

Besides the chilblains there is nothing unusual in the history of paroxysmal hemoglobinuria of this patient. We found that attacks could be produced by exposure to cold. If the patient placed both hands in ice cold water for five minutes no hemoglobinuria followed. Sitting before an open window for fifteen minutes, clad in hospital garb, when the out-door temperature was about 10 degrees C., was followed by a characteristic chill, fever and hemoglobinuria. If one foot was placed in a pail of water, 8 degrees C., for thirty minutes an attack would follow. During the interim the patient's blood showed a coagulation time of three and one-half minutes. Directly after an attack, while the temperature was still elevated, the coagulation time was the same.

On March 3, 1908, the patient's blood-count was the following:

| | |
|---|---|
| W. B. C., 7,800. | R. B. C., 4,520,000. |
| Hb. (Sahli), 92. | Tallquist, 85. |

*Read in the Section on Practice of Medicine of the American Medical Association at the Fifty-ninth Annual Session, held at Chicago, June, 1908.

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A differential count of 200 white cells gave the following results:

| | | |
|---|---|---|
| Polymorphonuclear cells, | 158 = | 79 per cent. |
| Small lymphocytes, | 31 = | 15.5 per cent. |
| Large lymphocytes, | 8 = | 4 per cent. |
| Eosinophiles, | 3 = | 1.5 per cent. |

Repeated search for blood parasites proved negative, as did also all attempts to cultivate any organisms from the blood by the usual methods employed for such purposes. These examinations were made during the attacks and during the interims. On March 13 an attack was produced by placing the patient's foot in a pail of water at 8 degrees C. The following observations were made before, during and after the induced attack of hemoglobinuria.

The patient's foot was placed in the water at 10:50 a. m. Although there were some subjective premonitory signs of a chill at 11:50 it was not until 11:55 that the rigor was pronounced.

The temperature by mouth was:

At 10:20 a. m., 98.3 degrees.

At 11:20 a. m., 97.6 degrees. Subjective symptoms of muscular soreness.

At 11:50 a. m., 97.4 degrees. Chill apparent.

At 12:24 p. m., 100.2 degrees. Patient felt warm; urine passed rich in hemoglobin.

At 12:45 p. m., 102 degrees.

At 1:20 p. m., 100.2 degrees.

The arterial blood pressure was 146 mm. Hg., systolic pressure, before, during and after the attack.

The diastolic arterial pressure during the corresponding periods was 95. The chill and fever did not, therefore, modify the systolic or the diastolic blood pressure.

During the febrile period at 12:50 p. m. blood drawn into a syringe containing a small amount (2 per cent. of a 1 per cent. solution) of potassium oxalate and immediately centrifuged showed a distinctly pink serum which gave only the absorption bands of oxyhemoglobin to spectroscopic examination. Blood drawn and treated in the same manner before the patient's foot was placed in the cold bath revealed a normal serum. A few days later a cantharides plaster was applied to the arm. Three hours later when the serum was expected to accumulate in the underlying blister an attack of hemoglobinuria was induced. This serum was pink and showed the oxyhemoglobin bands under the spectroscope. A cantharides blister during an interim showed straw-colored serum.

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The leucocyte count before an induced attack was 6,000; at the end of the chilly period the leucocyte count was 17,000 and was due to an increase of the polymorphonuclear cells. The leucocyte count made on March 7 was as follows:

| | |
|---|---|
| At 9:00 a. m., before the chill, | = 6,000 |
| At 11:30 a. m., after the chill, | = 17,000 |
| At 1:00 p. m., | = 15,000 |
| At 4:00 p. m., | = 11,000 |

During this attack the red cells were reduced 700,000 per c. mm. An ophthalmoscopic examination during one of the attacks revealed nothing abnormal in the eye-ground.

During the attacks the urine was colored a dark chocolate hue and showed nothing more than the presence of albumin, hemoglobin and casts of blood pigment.

From the above observations it was apparent that exposure to cold caused hemoglobinemia which was well pronounced before the febrile period. It is also clear that the rigor is associated with a slight lowering of the body temperature, and that the rise in temperature marks the cessation of the chill, which is unlike the relations between rigor, temperature and sense of warmth in the course of infectious diseases with which we are familiar.

The rise in temperature is associated with a pronounced polymorphonuclear leucocytosis.

The second patient B, an American, 25 years old, was admitted to Lakeside Hospital April 18, 1908. This patient was much more sensitive to cold than patient A. Without any history of illness or infection of any kind which could have any bearing on his present illness, B commenced having paroxysmal attacks of hemoglobinuria identical in character with those of A, eighteen months before admission to the hospital. As in A, the physical examination revealed absolutely nothing of an abnormal character. During warm weather he is perfectly comfortable, but if the thermometer is as low as 10 C., he is liable to an attack. He is particularly sensitive to a cold, moist wind, and has had as many as three attacks in one day. This patient gave no history of chilblains as did A, but at the conclusion of an attack there would be on the trunk and arms several small macules about one-sixteenth of an inch in diameter, in the middle of which a small blister would rise, from which the serum would escape during the febrile period and leave a very minute particle of desquamating epithelium apparent in the middle of the red

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areola. By the following morning all traces of the skin manifestations were gone.

The observations made on patient A were repeated on patient B. B suffered from a malady identical in character with that of A; the only essential difference being one of degree.

### STUDY OF THE SERUM IN THESE CASES.

We then undertook a study of the serum of these patients from the standpoint of the side-chain theory, with the following results:

Although we were unable to find any organism in the patient's blood, either during the attacks or in the interims, it was clearly apparent that cold was essential for the hemolytic activation of the patient's blood.

Our first experiments consisted in attempting to activate the serum of these patients by exposing it to a low temperature, then warming the serum to 37 degrees C., and adding washed red-blood cells obtained from other patients with normal blood. After repeated trials with serum of these hemoglobinuric patients obtained by venous puncture and from blister serum (all of which were unsuccessful as long as the red cells were added to the serum when warm), we then chilled the serum after red cells had been added. When this precaution was taken, and the mixture warmed, hemolysis was complete within a very brief period.

All the experiments gave the same results with sera A and B.

Hemoglobinemic serum chilled in a salt and ice mixture at 5 degrees C., for thirty minutes, then put in an oven at 37 degrees C. for one hour, and after the serum again attained body temperature the red cells from a normal blood which had been repeatedly washed in physiologic salt solution were added to the serum and again put in the oven. The result was negative. The experiment and results are expressed in the following formula:

H. S. + 5° C. for 30 minutes + 37° for 1 hour + R. B. C. = 0.

In the next experiment the red cells were added before the mixture was exposed to a low temperature, and as soon as the mixture was again warmed complete hemolysis occurred. This was apparent by the disappearance of all cloudiness and the mixture becoming a clear ruby-red solution with a marked diminution of the viscosity of the serum.

H. S. + R. B. C. + 5° for 30 minutes + 37° C. for 10 minutes = +.

The serum from patient B, with added red cells, was placed in the ice water for fifteen seconds, then put in a water bath heated to 56 degrees C. for ten seconds, at the end of which time hemolysis was complete.

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The serum from patient A required a longer exposure to cold to produce hemolysis, which is quite consistent with the comparative clinical histories of the two men. B was much more sensitive to cold than A.

Accidentally we discovered that if the suspension of red cells in the serum of B was placed near a 32-candle-power incandescent electric lamp after having been exposed to cold, hemolysis occurred very promptly.

If a suspension of red cells in the serum was placed in a water bath at 15 degrees C. for five minutes and then exposed to the rays of the lamp, which registered 25 C. on a thermometer placed beside the test-tube, hemolysis was prompt and complete, that is, it occurred at the end of eight minutes.

A drop of the suspension of red cells in serum from B was placed under the microscope after the mixture had been exposed to cold. The rays of an Abbé condenser supplied sufficient heat to cause rapid hemolysis, which could be observed under the microscope. Many cells disintegrated by fragmentation and many faded from view as the hemoglobin content was dissolved out of the stroma. The entire field was then filled with fragments of red cells which would certainly have been mistaken for blood platelets so far as their morphology was concerned.

If the suspension of red cells in serum A or serum B were exposed to a low temperature for hours, no hemolysis occurred until the mixture was again warmed.

The reduction in temperature necessary in the laboratory tests to procure activation of this lytic serum gives a very striking suggestion of the radiation of heat from the surface of the body and the subsequent warming of the blood from the surface, as it again meets the visceral blood, and it also illustrates the maintenance of the internal heat by metabolism.

If serum from normal blood was substituted for the lytic serum, and washed red cells from either A or B were substituted for the normal red cells employed in the former experiments, hemolysis did not occur.

This showed that the lytic principle was contained in the serum, and that hemolysis did not result from a diminished resistance of the patient's red blood cells.

If to a suspension of washed red cells (5 per cent.) in 0.5 c.c. normal serum, 0.03 c.c. of serum B was added it was found to be the minimum proportion of lytic serum necessary to cause hemolysis. So it may be said that approximately 6 per cent. of serum B added to normal serum was required to cause hemolysis of 5 per cent. suspension of red cells.

Thus far it is evident that cold (15 degrees C.) is essential for fixation of the lytic principle to the red cells, and that a temperature of 25 C. is necessary for the accomplishment of hemolysis.

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This behavior of the lytic principle strongly suggests the applicability of the side-chain theory to this instance of hemoglobinuria.

In an article by Donath and Landsteiner[1] we find they had the same experience as we had in producing hemolysis *in vitro* with the serum from a case of paroxysmal hemoglobinuria, and, although they attempted to prove the applicability of various steps in the side-chain theory, they failed to reactivate the serum after it had been inactivated by heat at 56 degrees C. for ten minutes.

If the serum is heated to 56 C. for twenty-five minutes all attempts to procure hemolysis by exposure to cold and then to 37 C. are futile. This shows, of course, that there is an unstable substance essential for hemolysis which answers to the complement of Ehrlich's theory. It now remains to be shown that there exists also a stable substance which serves as the amboceptor between the complement and the red cells. The first step to prove this point requires a successful reactivation of the serum. If done by the usual method employed in hemolytic experiments as tried by Donath and Landsteiner the result is negative.

0.5 c.c. of inactivated H. S. + washed R. B. C. + 5° C. for 30 minutes + 37° C. for one hour.

Now if the normal serum be added and the mixture is placed in the thermostat for many hours no hemolysis follows.

Inasmuch as a low temperature was required to procure fixation of the amboceptor to the red cell it seemed probable that reactivation experiments had hitherto failed because a low temperature was not employed in fixing the complementophilic end of the amboceptor to the haptophore of the complement. The following reactivation experiment proved successful.

0.5 c.c. of inactivated H. S. + washed R. B. C. + 0.25 c.c. of normal serum + 5° C. for 15 minutes.

The mixture was then warmed to 37 degrees C. and hemolysis followed immediately.

Thus it is apparent that cold is essential for the fixation of both the cytophilic and the complementophilic ends of the amboceptor.

The foregoing experiments prove the existence of two essential factors in the lytic principle of the serum, one of which is unstable and is rendered inactive by heat and is present in all human sera; the other factor is more resistant to heat and is present only in the serum of patients in whom exposure to low temperature produces hemoglobinemia.

Eason[2] in his experiments on the serum of a patient with paroxysmal

1. Donath and Lundsteiner: München. med. Wchnschr., 1904, li, 1590.
2. Eason: Jour. Path. and Bacteriol., 1906-7, xi, 169.

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hemoglobinuria by a different procedure showed practically the same point, although he failed to recognize the need of a low temperature to secure union between the complement and amboceptor. In his experiment red blood cells were added to the patient's inactivated serum and the mixture exposed to ice-water for half an hour. After the mixture was removed from the ice-water bath the complement from normal serum was added, how long after he does not state, but from our results the mixture must have been cold when the complement was added or hemolysis would not have occurred when the whole mixture was put in the brood oven.

If we are permitted to use the terms amboceptor and complement as applied to these two factors contained in the lytic serum it is incumbent on us to show that the union between the amboceptor and red cell is a biochemic union and one that can not be destroyed by washing the cells with physiologic salt solution after red cells have been exposed to cold in the presence of the inactivated serum from patients A and B respectively. At this period in our work we found that the oxalate serum did not differ from the serum which was procured by letting the serum separate out of the blood on standing. And as the serum could be procured more promptly and in larger amounts by centrifuging the oxalate blood, hereafter when the term H. S. is employed we shall mean hemoglobinemic serum to which 2 per cent. of 1 per cent. solution of potassium oxalate has been added.

The following is an experiment to show the fixation of the amboceptor which proved negative because the mixture was not exposed to a low temperature after the complement from a normal serum was added.

0.5 c.c. of inactivated H. S. + washed R. B. C. + 5° C.
for 1 hour.

The mixture was centrifuged and the supernatant fluid drawn off; the red cells were washed three times with large amounts of an isotonic salt solution. We then had presumably the red cells with the amboceptor fixed to them and clear of any of the excess of amboceptor which might have clung to the cells from the inactivated H. S.

If normal serum was now added to these (red cells + amboceptor), and the mixture placed in an oven at 37 degrees C. no hemolysis followed. In subsequent experiments, however, the mixture of (red cells + amboceptor) + normal serum was exposed to a temperature of 5 degrees C. for fifteen minutes and then hemolysis occurred as soon as the mixture was again warmed to 37 degrees C. Here again we have proof of the need of cold to procure fixation of the complementophilic end of the amboceptor to the haptophore of a normal serum.

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The following experiments were made to show how the amboceptor could be exhausted from the lytic serum.

To 1 c.c. of inactivated H. S., five drops of a 10 per cent. suspension of washed red cells were added. The mixture was exposed to 5 degrees C. for one hour, then centrifuged and the supernatant serum drawn off. To the supernatant serum, red cells were again added and then 0.25 c.c. of normal serum added to the mixture. This mixture was exposed to cold for fifteen minutes and warmed to 37 degrees C. Hemolysis followed immediately, but it was not so pronounced as in our other reactivation experiments.

If to 1 c.c. of inactivated H. S. five drops of a 10 per cent. suspension of red cells were added, the mixture exposed to 5 degrees C. for one hour and then centrifuged, the supernatant serum again exposed to 5 degrees C. for one hour with five drops of a 10 per cent. suspension of red cells again added to the serum, and then centrifuged, the supernatant serum could not be reactivated.

Thus we have shown that if in two stages, ten drops of a 10 per cent. suspension of red cells be added to (1 c.c.) the serum and exposed to cold for one hour each time, the amboceptor can be exhausted from the inactivated hemoglobinemic serum.

From a number of such experiments we learned that if the serum with red cells was exposed to cold less than one hour we could not procure fixation of the amboceptor.[3] Thus far we have shown that the hemoglobinemic serum contains two factors, one unstable and capable of being inactivated by exposure to a temperature of 56 degrees C. for twenty-five minutes and is a factor which is common to all human sera and answers to the complement of Ehrlich.

The other factor is found only in the hemoglobinemic sera and is resistant to 56 degrees C. for twenty-five minutes. This factor answers to the amboceptor of Ehrlich and can be fixed in a biochemic union to the red cells and can be exhausted from the hemoglobinemic sera if a sufficient number of red cells be suspended in the serum and the mixture exposed to a low temperature for one hour.

In watching hemolysis take place under the microscope and seeing the extreme rapidity with which hemolysis occurred in the test-tubes we were impressed with the striking similarity between this hemolysis and the hemolysis which occurs when small amounts of ether are added to a test-tube of isotonic salt solution with red cells in suspension.

3. Eason (Jour. Path. and Bacteriol., 1906-7, xi, 169) was able to procure fixation of the amboceptor after one-half hour exposure to cold. He was also able to exhaust the amboceptor by one-half hour exposure to cold.

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The natural deduction from such observations was that the lytic principle of the serum attacked the lecithin, cholesterin or some other lipoid of the red-cell stroma. If this were the case, then an excess of a mixture of lecithin and cholesterin in the serum would satisfy the avidity of this lytic substance and protect the red cells from destruction.

The following experiment was made to show this point:

An emulsion of lecithin (Merck) in normal salt solution and a mixture of cholesterin (Merck) in normal salt solution were made. Both mixtures were very thick. Three drops of each were added to 0.5 c.c. of H. S. and the mixture permitted to stand for one hour. Washed erythrocytes were added to the mixture and the whole mass exposed to 5 degrees C. for thirty minutes and then put in the oven at 37 degrees C. for twelve hours. No hemolysis followed.

We found then in trying lecithin and cholesterin separately that lecithin did not in the least inhibit hemolysis, but that cholesterin always prevented hemolysis even in the slightest degree when present to the extent of 5 per cent. or more.

It remained to be shown how cholesterin exercised this inhibitory effect on hemolysis—whether cholesterin exercised merely a negative reactive influence or formed a union with the complement or with the amboceptor.

To the hemolytic serum an emulsion of cholesterin in normal salt solution was added, and the mixture permitted to stand in the oven at 37 degrees C. for one hour. The mixture was then centrifuged and the clear supernatant serum removed. To this clear serum cells were added and the suspension of cells in the serum put in the cold bath at 5 degrees C. for one hour. The mixture was then put in the oven at 37 degrees C. and at the end of twelve hours there was no hemolysis.

From this experiment we are permitted to assume there was a deflection of either the amboceptor or the complement. Unlike the behavior of the receptors of the red cells and the amboceptor, however, we found by repeated experiments that a low temperature was not favorable to anchorage of the cholesterin.

Cholesterin protected the red cells when 5 degrees C., 22 degrees C. and 37 degrees C. were employed, but the best protection seemed to be afforded when the cholesterin was in contact with the serum at 37 degrees C., so the affinity between the lytic principle of the serum and cholesterin was not identical in character with the linking of the amboceptor to the red cells.

The following experiments were then made to show whether the amboceptor or the complement was deflected:

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One c.c. of the serum was inactivated by heating it at 56 degrees C. for twenty-five minutes. To this inactivated serum cholesterin in excess was added and the mixture permitted to stand at 37 degrees C. for one hour. The mixture was then centrifuged and the supernatant serum was clearer than the original unheated serum. We then assumed if cholesterin was fixed in this inactivated serum it must be fixed to the amboceptor.

To this clear serum washed erythrocytes were added and the suspension was exposed to 5 degrees C. for one hour. The mixture was then warmed to 37 degrees C. and 0.4 c.c. of normal serum was added and the mixture again chilled at 5 degrees C. for one hour. The mixture was then put in the oven at 37 degrees C. and in thirty minutes hemolysis was complete.

Therefore, treating the inactivated serum with cholesterin did not prevent anchorage of the amboceptor to the red cells.

The following method was then employed to show whether cholesterin deflected the complement or not in a reactivation experiment:

One c.c. of H. S. was heated at 56 degrees C. for twenty-five minutes. Red cells were then added and the mixture chilled at 5 degrees C. for one hour to fix the amboceptor. Cholesterin was then added to the mixture after it stood in the oven at 37 degrees C. for twenty minutes. Normal serum was then added to the mixture which was chilled at 5 degrees C. for thirty minutes. The mixture was then warmed and permitted to stand until the following day, but there was no hemolysis. This experiment shows that cholesterin acts by deflecting the complement.

Early in our experiments on these patients we tried calcium lactate as a remedy against hemolysis in both patients. They were given sixty grains a day over a period of two weeks, but at the end of that period the patients responded to cold as before and the hemolysis *in vitro* was unaffected. Nor did calcium lactate inhibit the hemolysis *in vitro*. After our experiences with cholesterin in the laboratory we administered cholesterin to these patients per os ten grains three times daily dissolved in olive oil. After ten days' trial of the cholesterin treatment we found that hemolysis *in vitro* was unaffected in both patients. The weather during this period was very warm, so neither patient had an attack, and although neither patient objected to repeated venous punctures for obtaining blood, they did not wish to expose themselves to an attack of hemoglobinuria by exposure to a cold foot bath. So whether the cholesterin therapy may offer protection against attacks due to exposure to cold or not remains undecided.

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We make this statement in view of the experience which Widal and Rostain[4] had with their patients, on whom they were able to confer passive immunity by means of an antihemolytic serum. Although the patients of Widal and Rostain enjoyed immunity from attacks on exposure to cold, their sera was as actively hemolytic *in vitro* during this period of immunity as before.

We repeated the experiment of Widal and Rostain in procuring an antihemolytic serum from a rabbit by treating the animal with normal human serum.[5]

The rabbit was given four intraperitoneal injections during a period of three weeks. The amounts of normal serum used at each injection were 5 c.c., 15 c.c., 12 c.c. and 12 c.c. respectively.

To inhibit the lytic action of these patients' sera on red cells we found it necessary to employ about 12 per cent. of the inactivated immune rabbit's serum added to the serum of patients A and B. Normal human serum was hemolytic to the rabbit's washed red cells, but the sera from our patients was more strongly hemolytic than normal human serum.

In tube A was placed 10 c.c. of normal human serum and in tube B was placed 10 c.c. of hemoglobinemic serum from patient B. To each, i. e., A and B, were added 1 c.c. washed red cells from the rabbit. Both tubes were chilled at 5 degrees C. for twenty minutes and then both tubes were put in water (55 degrees C.) for fifteen seconds. Both tubes showed hemolysis immediately, but the hemolysis in tube B was more complete than in tube A. We also found that normal human serum completely laked the rabbit's cells at 37 degrees C. in forty-five minutes without the previous exposure to cold.

The treated rabbit's serum also completely laked normal human red cells at 37 degrees C. in forty-five minutes without previous use of cold.

To test the antihemolytic property of this "sensitized" rabbit serum it was, of course, necessary to inactivate the serum at 56 degrees for twenty-five minutes.

We then found it was necessary to add as much as 12 per cent. of the inactivated rabbit's serum to the serum of patient B before hemolysis could be completely prevented. The antihemolytic property of our rabbit's serum was not so highly developed as in the rabbit of Widal and Rostain, probably because the rabbit was treated for a shorter time than was the rabbit in the hands of Widal and Rostain.

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4. Widal and Rostain: Compt. rend. Soc. de biol. de Paris, 1905, 1, 321.

5. Eason (Jour. Path. and Bacteriol., 1906-7, xi, 203) employed the serum of a patient suffering from paroxysmal hemoglobinuria for the same purpose. Any human serum serves the same purpose.

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Widal and Rostain found that they could confer a passive immunity on their patients by subcutaneous injection of 25 c.c. of the inactivated serum from the sensitized rabbit. This immunity against cold lasted for only ten days. But during the immune period the lytic property of their patients' serum *in vitro* was undiminished.

This antihemolytic serum of the sensitized rabbit inhibits the hemolysis if it is added to the mixture of red cells and lytic serum after exposure to cold.

The protection from this serum is by virtue of deflection of the complement and is not due to antiamboceptor.

The question of etiology arises in relation to these experiments in hemolysis in the following manner:

1. Is the lytic property of these patients' sera due to a want of the normal antihemolysin which is presumed to be present in the sera of all normal animals?

2. Does the lytic principle of these sera partake of the character of a catalytic agent or a ferment?

3. Or is the lytic substance a biologic product of some form of infection which thus far has defied our attempts at detection?

If the first hypothesis were tenable we should expect to find hemolysis inhibited by the addition of inactivated normal human serum. This, however, is not the case. If inactivated normal serum is added to the fresh lytic serum in equal amounts and the mixture permitted to stand, either at a low temperature or at 37 degrees C. for an hour before red cells are added, hemolysis results after the mixture is exposed to cold just as in the original experiment.

The second hypothesis is not tenable because the amboceptor can be readily exhausted from the inactivated lytic serum by the exercise of its function of fixation to the red cells. A catalytic agent would not be sacrificed in such measured quantities by the exercise of hemolysis.

The third hypothesis does not admit of disposal. It is true that these patients are in good health as long as they are not exposed to cold, and the fact that they have hemoglobinemic fever can not be regarded as the direct effect of some organism. The abundance of cell shadows which must exist in the plasma of these patients after hemolysis occurs is quite sufficient to account for their fever. But patients may have infections without an elevation of temperature, and that, too, when organisms can be cultivated from their venous blood.

We had a patient in our wards at Lakeside Hospital during the past year who was a good example of an afebrile infection.

A young man 20 years old presented all the clinical signs of Hanot's

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type of hypertrophic biliary cirrhosis of the liver with splenic tumor and slight icterus. The patient remained three months in the ward and during that time a careful watch of his temperature never detected a rise above 99 degrees F. From the fluid gained by punctures of the spleen and the liver and from blood obtained from the vein of his arm we were able to cultivate a large motile bacillus with many characteristics of the common colon bacillus. It is true that the patient was ill, but he was not ill on account of his infection, but on account of his diseased liver and spleen. With such experiences in mind it does not require a great flight of imagination to conceive of an infection of some kind being the underlying cause of our patients' hemoglobinemia.

Now, if such were the case we should expect to find some evidences of an antibody in the blood of the patient who has had his disease five years and who is less sensitive to cold than the other patient who has had his disease only one and a half years.

With this idea in view, inactivated serum from patient A was added in equal amounts to the fresh serum of patient B and inactivated serum from patient B was added to the fresh serum from patient A in equal amounts. These mixtures were permitted to stand one hour before red cells were added, but hemolysis resulted after the usual procedure of exposing the mixtures to 5 degrees C. and 37 degrees C. as in our earlier experiments.

As the matter at present stands we are justified only in saying that the hemoglobinemia which follows exposure to cold is due to some biologic product in the plasma which in its behavior admits of the demonstration of every step in hemolysis as expounded by the side-chain theory of Ehrlich.

In conclusion it gives us great pleasure to express our gratitude to Dr. J. J. Macleod, professor of physiology in Western Reserve Medical School, for valuable suggestions and consultations during the progress of our work.

Rose Building.

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ERRATUM.—An absurd typographical error occurred in the title of Dr. Herrick's paper in the October issue of the ARCHIVES (p. 291), where the word "cure" occurred instead of "case."—EDITOR.

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