glands. She was caged with male dogs for a year and never became pregnant, but she is neither sluggish nor somnolent. The remainder of the series are eliminated because when killed for examination vestiges of hypophysis were found, or the dog died of pneumonia or meningitis soon after operation. The dogs with hypophysis remnants showed no symptoms of hypophyseal deficiency.

All the dogs (except No. 52) that died following the hypophysectomy operation, the cause of death was either meningitis or pneumonia (probably aspiration pneumonia). These dogs died within 3 to 10 days following the operation.

1. Completely hypophysectomized dogs may live indefinitely without showing any of the Fröhlich syndrome.

2. Some of the completely hypophysectomized dogs show Fröhlich's syndrome in varying degrees (retarded growth, adiposity, somnolence, loss of sex urge).

3. The fact that all completely hypophysectomized dogs do not exhibit the Fröhlich syndrome seems to indicate that the hypophysis itself is not the only factor involved in this malady. The obvious varying or uncontrollable additional factor is the injury to the base of the brain in these operations.

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The auto-hemolysin of paroxysmal hemoglobinuria.

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The essential characteristic of the condition known as paroxysmal hemoglobinuria is the occurrence, as a result of exposure to cold, of hemoglobinæmia and hemoglobinuria, usually accompanied by a chill and rise of temperature. In the blood of these patients there is present an auto-hemolysin which is readily demonstrated by the simple procedure well known as the Landsteiner reaction. In its simplest form it consists simply

in chilling the patient's blood to 0° C. for 30 minutes, and then warming it to 37° C. In normal blood no hemolysis occurs after the blood has been warmed to 37°. More consistent results are obtained by using serum and a suspension of washed red cells, and by adding complement. This auto-hemolysin has been the subject of a number of studies which need not here be reviewed. The reaction has been shown to be an antigen-amboceptor-complement reaction with the characteristic that the hemolysin unites with the red cells only at a low temperature. It has also been shown that the combination of corpuscles and hemolysin occurring at low temperatures is at least partially broken up at higher temperatures, and that an iso-hemolysin demonstrated by chilling, as well as the auto-hemolysin, is present.

During the past three years we have had an opportunity to study the blood of three patients who are subject during the winter months to typical attacks of hemoglobinuria. Unless otherwise stated, the Landsteiner reaction has been done in our work by using 0.25 c.c. of serum, and 0.1 c.c. of a 5 per cent. suspension of washed red blood cells, and adding as complement 0.1 c.c. of pooled guinea pig serum diluted 1 : 10. The volume is made up to 0.5 c.c. with normal salt solution, and the tubes are then kept at 0° C. for 10 minutes, and in the water bath at 37° for 2 hours, when readings are made. Control serums and red cell suspensions were always from the same blood group.

In contrast with most sensitizing antibodies, this auto-hemolysin may show marked thermolability. In one of our cases 45° C. for 30 minutes destroyed the hemolysin so that it could not be reactivated by addition of fresh complement. The temperatures required to destroy the hemolysin in the other two cases were 47.5° C. and 55° C.

The demonstration of the presence of the auto-hemolysin is usually accomplished by chilling the serum-erythrocyte-complement mixture to 0° C., but obviously such a low temperature is not present at the site of the *in vivo* reaction. It seemed worth while, therefore, to determine the highest temperature at which the union of hemolysin and erythrocyte occurs. Two of the hemolysins sensitized at temperatures from 0° C. to 12° C.; the third did not sensitize above 8° C. It seems quite possible that the blood in the superficial capillaries may, in cold weather,

be chilled to 12° C., or even 8° C. It is not unlikely, therefore, that in the spontaneous paroxysm, the same mechanism operates as in the test tube reaction.

Yorke and Macfie[1] recently reported that hemolysis is greater if the erythrocyte-hemolysin-complement mixture is chilled only 5 to 7 minutes, rather than 30 to 60 minutes. We have confirmed this observation. Their explanation of this somewhat paradoxical phenomenon is that, with longer exposures at the low temperature, the complement is distributed among a larger number of sensitized cells with the result that each cell has less complement, and some cells have ineffective quantities. That this explanation is not entirely satisfactory is evident from the following experiment. To graded red cell suspensions from 0.5 per cent. to 50 per cent. constant amounts of serum and complement are added. The mixtures are chilled and then warmed in the usual manner. It is found that the amount of hemolysis increases with the percentage of the cell suspension, and that suspensions of 0.5 per cent. and 1.0 per cent. showed no hemolysis. With heavier suspensions there would presumably be a distribution of complement among a larger number of cells, and yet there is more hemolysis in the heavy suspensions. It therefore seems unlikely that the explanation of Yorke and Macfie for the greater hemolytic effect of short chilling is correct.

One of the interesting questions in paroxysmal hemoglobinuria is the relation of this condition to syphilis. About 90 per cent. of these patients as reported in the literature have had a positive Wasserman reaction. Many of them have been congenital syphilitics; others have had lesions of acquired syphilis, and some have had only serological evidence of syphilis. One of our three cases is a congenital syphilitic; another probably has syphilis of the liver, and the third has a history of syphilis but no demonstrable lesions at present. All three have positive Wasserman reactions, and it is of interest that the reaction in two of the three cases is extraordinarily strong. The highest titre was found in the congenital syphilitic, a girl of 11, whose Wasserman titration at .001 c.c. was: alcoholic antigen +++, cholesterin antigen ++++, larger quantities gave ++++ reactions with both antigens. The Landsteiner reaction was found

[1] Yorke, W., and Macfie, J. W. S., *Brit. J. of Exp. Path.*, 1921, ii, 115.

to be negative in four congenital syphilitics without paroxysmal hemoglobinuria, all of whom had strong Wasserman reactions.

If the serum of the hemoglobinuric is exposed to an equal volume of a 100 per cent. suspension of his own erythrocytes at 0° C., and the mixture is centrigugalized cold, the supernatant serum is found to have lost the Landsteiner auto-hemolysin. It has been completely absorbed at the low temperature. Wasserman reactions done on absorbed and unabsorbed serum show an almost identical titre. It is, therefore, clear that the Landsteiner auto-hemolysin and the Wasserman reacting substance are distinct serological entities. The former may be removed without weakening the latter. This result harmonizes with the observation that of our three cases, the one having the highest titre of Landsteiner auto-hemolysin had the weakest Wasserman reaction.

Since the serum of the hemoglobinuric, in addition to the auto-hemolysin, contains a similar hemolysin for the erythrocytes of other individuals even of the same blood group, an attempt was made by absorption experiments to separate the auto-hemolysin from the iso-hemolysin. It was found that the red blood cells of other individuals, as well as the patient's own erythrocytes, absorbed out both the auto- and the iso-hemolysin. Presumably, therefore, they are inseparable and possibly identical.

It appears, therefore, that in this disease we are dealing with a thermolabile hemolysin which unites with its antigen only at a low temperature, that the union is more effective with chilling for only 5 to 10 minutes than with longer exposure to the low temperature; that complement is necessary for consummation of the reaction, that there is an intimate relation between this disease and syphilis, but that the substance upon which the diagnostic test tube reactions depend are not identical.