in the autoclave for a time, depending on the bulk of medium in preparation. While it is sufficient to have a litre flask containing 200 c.cm. of medium for 10 minutes at 1 atmosphere, a 5-litre aspiration bottle filled with medium requires three-quarters of an hour at 1 atmosphere pressure. It is advisable to add to the medium 10% of water to allow for evaporation in the autoclave.

This medium, when ready, is of a dark-brown colour. The addition of 1/2% of activated charcoal clarifies it to a certain extent. Fortunately, the waste water, after extraction of penicillin with amyl acetate, retains the bulk of the pigment, so that ultimately a powder is obtained with a degree of freedom from pigment corresponding to the stage of purification. Nor are these pigments, or other impurities derived from the medium, toxic: 1/2 c.cm. of sodium salt of 2,500 units per c.cm. injected into three mice did not seem to affect them adversely.

The titre of the crude penicillin solution obtained by using this medium, tested by serial dilution or ring method, frequently exceeds 60 units per c.cm. The average titre is above 30 units per c.cm. The highest figures obtained by me when using the Challinor and McNaughton medium were in the neighbourhood of 25 units per c.cm. I did not get up to this level with the Czapek-Dox medium.

Discussion

Apparently a nutrient medium prepared in the way described, besides containing all the glucose and most of the nitrogenous foodstuffs needed by a growing mould, must contain certain activating substances. By chemical analysis the nutrient medium was found to contain all the inorganic salts entering into the composition of Czapek-Dox salts except $MgSO_4$ and $NaNO_3$. The addition of $MgSO_4$ was shown by comparative experiments to be unnecessary, while $NaNO_3$ definitely improved the titre of penicillin. Corn-steep liquor certainly has a beneficial effect. In comparative experiments, while a flask containing hydrolysed potato medium would show at its height a titre of 15–20 units per c.cm., a flask containing medium to which corn-steep liquor has been added would show 60 and up to 80 units per c.cm. Before sterilization the addition of a small percentage of pulp, made from cabbage, or particularly carrots, seems to have a beneficial effect. As the height of titre constantly varies from day to day, and from experiment to experiment, I have not yet been able to draw definite conclusions as to the effect of such additions.

I need not enter into details of the actual cost of my medium. The economy is obvious—always on condition that the titre should not be lower than that of the other media used, and it does not seem to be so.

At present I have three experimental vessels—apparatus for penicillin production by surface growth in a continuous process—working at Highgate Hospital laboratory. From the seventh day—that is, from the time of the titre approaching its height—the average titre of all three tanks is 34.5 units per c.cm. What is the cost of this medium per litre? It amounts to the cost of 100 c.cm. of corn-steep liquor, 3 g. $NaNO_3$, 1 g. activated charcoal, and the potato peelings, which I get either from a hospital kitchen or from any canteen in the neighbourhood—free of charge. Working on a large scale, the additional cost would be the transport of potato peelings or diseased potatoes to the factory. True, on a farm one may use the peelings for feeding pigs, but the market value of it would hardly exceed 10s. a ton. One must therefore expect that agricultural waste will ultimately play an important part in the preparation of media for the production of various antibiotics.

I am much indebted to Dr. J. M. Alston for his constant interest and help in my work on antibiotics, and I must thank the London County Council and Dr. J. R. Reed for enabling me to work at Highgate Hospital.

The Federation of the British Legion has approved a recommendation of the Council of Management of the British Legion Village for the appointment of 21 medical men as honorary consultants to Preston Hall, Maidstone, Kent; the British Legion Sanatorium, Nayland, Colchester, Essex; and Douglas House, Bournemouth, the convalescent seaside annexe of Preston Hall. During the absence of Dr. J. B. McDougall (medical director of the British Legion Village), who is now attached to U.N.R.R.A., Dr. Frederick Heaf has been appointed honorary consulting medical director. The consultants will form a medical committee under the chairmanship of the honorary consulting medical director to advise the Council of Management in all medical matters connected with the British Legion Village.

HYPERSENSITIVITY TO TRANSFUSED BLOOD

BY

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AND

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A patient who is believed to be suffering from the rare disease called lupus erythematosus diffusus required a series of transfusions on account of persistent anaemia. As a result of these transfusions a remarkable succession of antibodies has appeared in her serum. The patient's red cells are Group O, M, P, Rh₁Rh₂, and negative with the three "new" antibodies to be described. She has never been pregnant.

Transfusions

Date	Donor's Name	Donors' Groups			
		Rh	Anti-Lutheran	Anti-Willis	Anti-Levay
1 Feb. 2, 1944	Marshall	Rh ₁ rh	—	—	—
2 Aug. 19, 1944	R. B.	rh rh	—	Unknown	—
3 Aug. 19, 1944	Lutheran	rh rh	+	—	—
4 Sept. 23, 1944	Willis	Rh ₁ Rh ₂	—	+	—
5 Sept. 29, 1944	Mules	Rh ₁ rh	—	—	—
6 Oct. 6, 1944	Howes	Rh ₁ Rh ₂	—	+	—
7 Nov. 7, 1944	Levay	Rh ₁ Rh ₂	—	—	+
8 Nov. 20, 1944	Allington	Rh ₁ Rh ₂	—	—	—
9 Jan. 31, 1945	"	Rh ₁ Rh ₂	—	—	—

Anti-Lutheran = The patient's serum taken at the end of Aug., 1944.

Anti-Willis = The patient's serum taken in Oct., 1944.

Anti-Levay = The patient's serum taken in Nov., 1944.

A specimen of serum taken shortly after the Lutheran transfusion was found to contain the rh agglutinin called St (γ Fisher); the "incomplete" form of this antibody was also present. More interesting, however, was the finding of another separable agglutinin, called anti-Lutheran in the table. The red cells of 8–9% of the English population, irrespective of the ABO, MN, P, or Rh groups, are agglutinated by this antibody. The Lutheran antigen is inherited as a dominant Mendelian character. The statistical and family evidence will be presented in detail elsewhere.

The St and anti-Lutheran antibodies gradually disappeared, their titre being low at the time of the September and October transfusions. After the Willis, Mules, and Howes transfusions a second "new" antibody—anti-Willis—appeared. The donor Howes's blood presumably assisted in this immunization, for Howes also has the Willis antigen. The Willis antigen is inherited as a dominant Mendelian character independent of ABO, MN, or P but not independent of Rh, and it is now practically certain that it is controlled by another allele at Fisher's C-c locus (Race, 1944), which may be called C^w. The Willis antigen is present in about 7% of Rh₁Rh₂ bloods, and the relative frequencies of the three alleles in the English population must be about C 43.5%, C^w 1.5%, and c 55%. Again the evidence for this interpretation will be presented elsewhere.

After some weeks of existence anti-Willis faded from the patient's serum, but towards the end of this period Levay's blood was given. This blood also proved to be incompatible, for about 14 days later a weak agglutinin—anti-Levay—was evident. The Levay antigen is a rare one and probably also a "dominant," for so far it has been found only in the blood of Levay and in the blood of this donor's father, but not in several hundred random bloods examined. All three "new" antibodies are more active at 37° C. than at lower temperatures.

Attempts to immunize one volunteer to the Lutheran antigen and two to the Willis have so far had no success. As the Lutheran and Willis antibodies may not be reproducible, and as the supply of antiserum is limited, it is proposed to deposit the names and addresses of as many of the Lutheran and Willis positives as are willing to help at the Bureau of Human Heredity, 115, Gower Street, London, W.C.1. It is hoped with the help of Major Moloney, of the U.S. Army Medical Corps, to make some equivalent arrangement with people whose homes

are near New York. If either of these antibodies do turn up again, as they are sure to do sooner or later, it may be possible to correlate the present work.

Comment

There are two points of general interest in this history. First, the patient must be extraordinarily sensitive to antigenic differences, and this supports the hypothesis that the symptoms of the disease lupus erythematosus are manifestations of some undetected antigen-antibody reaction. Secondly, given such a hypersensitive recipient, a glimpse is obtained of the multiplicity of red cell antigens which may be found in the future. One familiar and three "new" antibodies have been made to the blood of eight donors, although considerable care has been taken to use appropriate blood. Landsteiner was of the opinion that blood groups would some day be known to be as individual as finger-prints.

Our thanks are due to Prof. Cappell and Prof. Fisher for continued interest and encouragement; to the donors for permission to use their names; and to the patient, who has been very co-operative.

REFERENCE

Race, R. R. (1944). *Nature*, **153**, 771.

A CASE OF NOCTURNAL HAEMOGLOBINURIA

BY

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The syndrome of paroxysmal nocturnal haemoglobinuria with haemolytic anaemia was first recorded by Marchiafava and Nazari in 1911, and according to Wintrobe only 41 cases had been described in the literature up to 1943.

Case Report

The patient, a Royal Marine aged 27, was admitted from a naval hospital for special investigation to the wards of Prof. J. W. McNee in the Western Infirmary, Glasgow. He was complaining of anaemia, with frontal headaches, tiredness, and weakness. He entered the Navy 8 years ago and his only illness was in Oct., 1943, while in the Faroe Islands. It was characterized by diarrhoea, vomiting, and urticaria, which was diagnosed as food poisoning. He has never had malaria. He is married and has one healthy child aged 21 months. There is no history of anaemia or jaundice in the family.

In March, 1944, the patient began to suffer from frontal headaches while working. These persisted unless he sat down and rested for a short while. These headaches gradually increased in severity, and he noticed that he was becoming very easily tired and breathless on exertion. In May, 1944, he reported sick, and was admitted to a naval hospital, where he was found to be profoundly anaemic. Three weeks later he developed a painful tongue and tender bleeding gums. The condition yielded rapidly to large doses of ascorbic acid, and glycerin and borax applied locally. For the anaemia he received iron and a variety of liver preparations without any improvement. When he had been two months in hospital he was given one pint of blood, with slight improvement in haemoglobin level. A month later he was given another pint of blood of correct group, after which, for the first time, his urine was noticed to be very dark in colour for two days following the transfusion. He was admitted to the Western Infirmary on Dec. 9 for special investigation, as the exact diagnosis of the anaemia remained uncertain.

On admission he was seen to be a pale well-nourished man with a slight icteric tinge of the skin and sclerotics; a few discrete hard lymphatic glands were present in both axillae and groins. The heart, lungs, and nervous system showed no abnormality. The liver and spleen were not palpable.

Blood Picture.—The following investigations were made, using the technique described by Wintrobe. Haemoglobin was estimated by means of a photo-electric colorimeter. Erythrocytes, 3,060,000 per c.mm.; leucocytes, 2,400 per c.mm.; haemoglobin, 11.4 g. per 100 c.cm.; packed-cell volume, 32.5%; mean cell volume, 106 c. μ ; mean corpuscular haemoglobin concentration, 34.8%; reticulocytes, 12.5%. Differential leucocyte count: neutrophils, 44.5%; eosinophils, 2.5%; lymphocytes, 48%; monocytes, 4%. A film stained with Leishman's stain showed anisocytosis, slight poikilocytosis, and macrocytosis of the erythrocytes. There was no evidence of malaria parasites or pigmentation of leucocytes.

On sternal puncture marrow was easily obtained; it appeared to be hypoplastic, as the film made from it contained numerous fat spaces. A few primitive erythroblasts were seen, but the marrow was definitely normoblastic. The blood plasma gave a positive indirect van den Bergh reaction—0.7 mg. of bilirubin per 100 c.cm. The fragility of the patient's erythrocytes to saline was within normal limits, haemolysis starting at 0.45%. Wassermann reaction, negative. A fractional test meal showed a normal hydrochloric acid curve. The urine contained a quantity of urobilin. At this point a haemolytic anaemia was diagnosed, the cause, however, being unknown.

Progress.—One week after admission the patient complained of pain in one of his lower molar teeth. The tooth was extracted under local analgesia on Dec. 18. At 2 a.m. on Dec. 19 he awoke and complained of an ache across his lumbar region, and a specimen of urine passed at this time was noticed to be dark red. Another specimen of urine at 6 a.m. was still dark in colour, while one at 9 a.m. was perfectly clear. The van den Bergh reaction on Dec. 19 showed a positive indirect reaction—1.4 mg. of bilirubin per 100 c.cm. Early on the morning of Dec. 21 he again passed a dark-red specimen of urine, while at 9 a.m. the urine was clear. Examination of the urine showed the colour to be due to a haemoglobin derivative. No erythrocytes were seen. The haemoglobin derivative was identified spectroscopically as oxyhaemoglobin. Since then haemoglobinuria has recurred regularly, the specimen passed in the early hours of the morning being dark red, that passed about five hours later being perfectly clear.

Further Investigations.—(1) The Donath-Landsteiner test was performed in order to exclude haemoglobinuria due to cold; it was negative. (2) In order to determine whether the fall in the pH of the blood during sleep was a factor in the production of the haemolysis (Ham, 1939) the patient's erythrocytes and serum were set up against an isotonic system over a pH range of 6.5 to 7.0 by Dr. W. B. Kyles, clinical pathologist to the Infirmary. The result was quite negative. (3) Even the clear specimens of urine contained a constant deposit of haemosiderin, demonstrated by the hydrochloric acid-potassium ferrocyanide reaction (Wintrobe). (4) As necrosis of the liver is a feature of this disease (Scott, Robb-Smith, and Scowen, 1938) liver function was estimated by the laevulose-tolerance and Quick's sodium benzoate tests, but was found to be within normal limits. (5) A specimen of serum was examined by Prof. D. F. Cappell; this identified the patient as Group O and Genotype Rh,Rh₂. (6) In order to bring out the association of the haemoglobinuria with sleep, his sleep rhythm was altered and he slept through the day and remained awake at night. This caused the haemoglobinuria to appear through the day while the patient slept, and was absent at night when he remained awake.

Discussion

The pathology of this disease and the mechanism of production of the haemoglobinuria are fully discussed by Scott, Robb-Smith, and Scowen (1938). The hypothesis put forward for this explanation of the nocturnal haemoglobinuria is the alteration in the pH of the blood during sleep (Ham, 1939). While sleep undoubtedly is a factor, as seen by altering the sleep rhythm, the laboratory experiment carried out by altering the pH of a specimen of blood, while not conclusive, would tend to point against alteration of the pH being part of the mechanism, as the range of pH covered was much greater than would occur in the human body. It is known that transfusions and trauma are factors which increase or precipitate the haemolysis. This is well shown in this case.

Summary

A case of chronic haemolytic anaemia associated with nocturnal haemoglobinuria is presented. Blackwater fever, syphilis, and Lederer's anaemia were excluded. The patient was Rh-positive. The existence of the nocturnal periodicity of the haemoglobinuria was confirmed by altering the sleep rhythm, thus indicating that the patient suffered from the Marchiafava-Micheli syndrome.

I should like to thank Prof. J. W. McNee for his permission to publish this case.

REFERENCES

- Ham, T. H. (1939). *Arch. intern. Med.*, **64**, 1271.
Scott, R. B., Robb-Smith, A. H. T., and Scowen, E. F. (1938). *Quart. J. Med.*, n.s., **7**, 95.

The Mothers' Hospital of the Salvation Army in Lower Clapton Road, London, E.5, is entirely staffed by medical women. The report for 1944 is curtailed in the interests of economy, but space has been found for a foreword by Miss Margaret Basden, senior obstetric surgeon, who on the outbreak of war took up residence in the hospital. She writes in moving words of the fine spirit with which the nursing staff and patients and the fire-watchers endured the ordeal of air raids.