blastosis foetalis; those cases of congenital obstruction with anomalous development of the biliary tract or with other malformation such as transposition of the viscera are almost surely developmental. However, the fact that some of them appear to be associated with icterus gravis is of practical importance. It cannot be too strongly stressed that any family showing a history of congenital obliteration should be examined for evidence of rhesus-incompatibility. Only in this way can preparations be made to anticipate haemolytic disease occurring in any subsequent child.

Since obstructive jaundice from plugging of the bile ducts with inspissated bile does occur in some instances, we have recently started the practice of massage over the gall-bladder region, twice daily, in cases of icterus gravis as a prophylactic measure in the hope of avoiding impaction. This we accomplish by inserting the tips of the fingers gently but firmly under the right costal margin, with the child in a sitting position, and allowing it to cry or breathe out forcibly against them. It is hoped that by this method either an actual or a reflex contraction of the gall-bladder will help to maintain a flow of bile through the common bile duct and hence withdraw bile from the intrahepatic canaliculi.

Summary

Evidence is adduced which suggests that certain cases of so-called "congenital" obliteration of the bile ducts occur as sequelae of icterus gravis neonatorum. Various mechanisms are suggested to account for the obstructive element in this disease, and the importance of recognizing this feature of haemolytic disease is stressed.

We wish to express our thanks to Prof. T. F. Hewer for his advice and help in this work and for providing material from one of his cases, which appears in this series. We also wish to acknowledge our gratitude to Dr. Beryl Corner and Dr. F. W. Lewis for material from two cases, and to Mr. S. A. Edwards and Mr. R. Polding for technical assistance.

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THE Rh FACTOR AND BLOOD TRANSFUSION OBSERVATIONS ON A GROUP OF Rh-NEGATIVE INDIVIDUALS TRANSFUSED WITH Rh-POSITIVE BLOOD

BY

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This paper is concerned with Rh studies on 138 wounded American soldiers transfused with whole blood at the 163rd (U.S.) General Hospital. Of this group 18 were found to be Rh-negative; these cases were investigated for the presence of Rh antibodies and also in regard to the survival of Rh-positive cells in the circulation of Rh-negative individuals.

Methods and Materials

The techniques for Rh testing and for the detection of Rh antibodies were carried out according to the methods described by Taylor, Race, Prior, and Ikin (1942). The test for blocking antibody was that of Wiener (1944). Some of the Rh-testing serum was obtained from the Army Medical School, Washington, D.C., but the greater part of this work was carried out with potent human anti-Rh sera made available through the kindness of Dr. R. R. Race and the late Dr. G. L. Taylor, of the Galton Laboratory Serum Unit, Department of Pathology, Cambridge.

The great majority of the patients had severe wounds of the limbs which necessitated relatively long periods of hospital treatment, and all but a few cases were available for repeated

Rh tests. All except one patient (Case 5) had received Group O blood in advanced medical units before admission. Records of these transfusions were meagre, and consisted usually of a note on the amount of blood and the date administered. Nearly all the blood used for transfusions at this hospital was obtained from the Blood Bank of the Department of Pathology, University of Cambridge, and the Regional Transfusion Service (British) in Cambridge. In this blood, glucose-disodium citrate mixture was used as the preservative, and the blood was not stored longer than two weeks before administration.

In the forward medical units of the U.S. Army the blood used was supplied through the European Theatre of Operations Blood Bank. Some of this blood came from the U.S., preserved in Alsever's solution; the blood drawn in the European theatre from U.S. Army personnel was preserved in a citric acid-sodium citrate-glucose mixture.

Results of Studies

Of the 138 patients studied, 18 (13%) were Rh-negative. These Rh-negative cases may be considered in three groups.

Group I

The patients in this group were given known Rh-positive blood at this hospital. The observations on these 6 cases (Nos. 1 to 6) furnish the most important data in this investigation.

Case 1.—Owing to retained Rh-positive cells from a transfusion 30 days previously, the patient was considered to be Rh-positive. He was given 1,700 c.cm. of Group A Rh-positive blood, but two weeks after the last transfusions the Rh test was only moderately positive and there were many unagglutinated cells. On the 23rd day following the last transfusion very few Rh-positive cells were found, and a test on the 29th day showed a complete absence of Rh-positive cells. A febrile reaction occurred during the second transfusion at this hospital, but no evidence of haemolysis was observed in the urine or plasma. Since subsequent transfusions with the same type of blood gave no difficulty, it was assumed that this reaction was pyrogenic in nature. Tests for blocking and agglutinating antibodies were negative.

Case 2.—This patient showed no retained Rh-positive cells 41 days after a transfusion of known Group A Rh-positive blood. Since no tests were carried out at an earlier date it is not possible to say how much more rapidly the Rh-positive cells were eliminated from his circulation.

Case 3.—In this case the Rh test was weakly positive one month after 1,500 c.cm. of blood had been given in France. Transfused with 500 c.cm. of a known Group A Rh-positive blood, 8 days later there were but a few Rh-positive cells left, and 16 days after the transfusion no Rh-positive cells were detectable.

Case 4.—Owing to a transfusion 12 days before admission this patient was thought to be Rh-positive. He was transfused with 500 c.cm. of Group A Rh-positive blood; the Rh test 34 days later revealed no Rh-positive red cells. As in Case 2, earlier testing might have demonstrated more rapid elimination of Rh-positive red cells.

Case 5.—No transfusions were given previous to admission. He was found to be Rh-negative, but nevertheless was given 500 c.cm. of Group O Rh-positive blood. Three days later only a few scattered Rh-positive cell clumps were found, and on the 4th day after transfusion no Rh-positive cells could be detected. This case is of special interest in that no prior transfusion had been given, so that previous iso-immunization seems to have played no part in this phenomenon.

Case 6.—In an advanced unit two days before admission this patient had received 500 c.cm. of Rh-positive blood. The Rh test showed visible agglutination and he was given 1,000 c.cm. of Group A Rh-positive blood. After three transfusions the haematocrit reading did not rise to the expected level, and in preparation for further transfusions it was noted that the Rh test gave less marked agglutination than before and that at least half of the red cells were unagglutinated. During the following month a total of 3,000 c.cm. of Group A Rh-negative blood was given. The patient had no untoward reactions and there was no evidence of haemolysis in his plasma or urine after any of the transfusions. Within 10 days the bulk of the Rh-positive cells were eliminated, but small clumps of Rh₁ and Rh₂ cells persisted. The patient was completely negative, being of the genotype rrrh.* Repeated tests for blocking and agglutinating antibodies were entirely negative until 53 days after the last Rh-positive transfusion. On this occasion the patient's serum was tested by Diamond's (Diamond and Abelson, 1945) technique. A drop of oxalated whole blood, Group O Rh-positive, was placed on a slide and a small drop of the patient's serum was

* The Rh typing was carried out by Dr. R. R. Race.

mixed with it and allowed to stand at room temperature. Within five minutes a marked agglutination was visible on the slide. This finding was checked with the usual tube agglutination technique, and visible agglutination in dilutions of 1:2 with microscopical clumping at 1:4 could be demonstrated. No blocking antibody was found. Diamond's test, employed eight days before in this case, was negative.

Group II

This group of 4 patients (Cases 7 to 10) had received blood before admission, but Rh tests showed them to be negative although clumps of Rh-positive blood had been given.

In Case 7 a considerable number of Rh-positive cells were retained for 47 days; by the 54th day most were gone, and all Rh-positive cells had disappeared within 60 days of transfusion. In Case 8 a similar situation was found, with complete loss of Rh-positive cells within 58 days. In Case 9 Rh-positive cells disappeared within 28 days.

Case 10 did not, unfortunately, remain under observation very long. This patient had had 2,250 c.cm. of blood before admission. With the last transfusion, a note on the chart stated that owing to chills, fever, and urticaria the transfusion was stopped. On admission to this hospital 10 days later there was no icterus or impairment of renal function. The nature of this reaction was not established. Practically all Rh-positive cells had been eliminated and only a very few small Rh-positive cell clumps could be found 10 days after the last transfusion.

Group III

In the 8 cases comprising this group the Rh type of blood given before admission was not known, and since no Rh-positive cells were retained the findings were incomplete. Statistically the chances greatly favour Rh-positive blood having been administered to these patients, but owing to lack of data no conclusions are warranted in this group.

Discussion

The survival of transfused red blood cells is influenced by a number of factors. The age of the blood, the preserving solution, and conditions of storage are obviously important considerations. In this study the cases in Group I were given blood preserved in a disodium citrate-glucose solution; the blood was kept at 4° C., and no blood stored over 14 days was used. On two occasions fresh whole blood in citrate solution was given to cases in Group I.

The blood used in transfusions before arrival at this hospital was stored under less favourable conditions. It is possible that some of this blood preserved in Alsever's solution, or in glucose-citrate mixture, would survive for 60 days under proper conditions. However, with varying temperatures and the considerable handling necessitated by conditions in advanced medical installations, serious impairment in the ability of such stored blood to survive for normal periods must be admitted. Also, in contrast to studies of cell survival in normal controls (Callendar, Powell, and Wits, 1945), the recipients in all the cases under consideration were seriously wounded men. All had received sulphonamides and penicillin; many had some degree of sepsis. Obviously such complicating factors would not enhance the survival of transfused red blood cells. Therefore the rapid elimination of transfused red cells in certain cases in Group II and Group III might be attributed to non-specific factors rather than a phenomenon related to Rh or any other antigen-antibody mechanism.

However, the blood administered at this hospital to the cases in Group I was preserved and stored in a manner similar to blood which, in recent transfusion studies, survived satisfactorily for 60 days or more (Loutit, Mollison, and Young, 1943). For this reason observations on the survival of this blood are of considerable interest. In Cases 1, 2, 3, and 4 the transfused red cells were eliminated rapidly and the previous transfusions may have had a sensitizing effect in spite of the fact that no antibodies were detected.

Case 5 had been given plasma but no blood before admission to this hospital. Within 3 days following a transfusion of 500 c.cm. of Group O Rh-positive blood he had eliminated all Rh-positive cells. No reaction or evidence of haemolysis had developed. Dacie and Mollison (1943) observed a similar rapid elimination of Rh-positive cells in an Rh-negative recipient during experiments on the survival of transfused red cells.

They also noted this phenomenon in Rh-positive individuals, and Mollison (personal communication) is of the opinion that certain recipients have a peculiar ability, unrelated to iso-immunization, of eliminating transfused red cells.

In this group of Rh-negative cases repeated attempts were made to detect antibodies for the Rh factor. However, in only one case (No. 6) were antibodies discovered. In this patient, 53 days after the last Rh-positive blood was given, good anti-Rh agglutinins were found. If repeated observations had been carried out over a longer period of time antibodies for the Rh factor might have been discovered in other cases. Almost at the end of these investigations the important work of Diamond became available (Diamond and Abelson, 1945). Diamond found that even weakly agglutinating anti-Rh sera and sera containing blocking antibodies, when mixed on a slide with concentrated Rh-positive red-cell suspensions, gave a rapid and definite macroscopic agglutination. At the time only 3 Rh-negative cases remained in the hospital, but one of these (Case 6) gave a markedly positive reaction by Diamond's method.

In addition to detectable antibodies there are other more occult factors concerned in the destruction of Rh-positive cells in Rh-negative recipients. Wiener (1945) has pointed out that the biologic test will at times reveal a haemolytic process in a sensitized individual which cannot be demonstrated by *in vitro* tests. It is quite probable that other as yet unrecognized humoral products of sensitization may be discovered. Furthermore, it is not unreasonable to suspect that the haemoclastic process may be activated by a combination of both humoral and reticulo-endothelial sensitization. This aspect of the problem needs further investigation.

From a practical standpoint, in advanced medical units in wartime the problem of Rh-incompatibility must be disregarded. In general hospitals and other fixed installations a blood bank with available Rh-negative blood should be maintained. All Rh-negative patients should receive Rh-negative blood; this is especially important if blood has been previously given or if there is a possibility that multiple transfusions will be necessary. Diamond, in an excellent review on the clinical aspects of the Rh factor, points out the danger of iso-immunization by repeated blood transfusions, especially in the treatment of war wounds. In Rh-negative patients given Rh-positive blood difficulty may be encountered in deciding whether the patient is actually Rh-positive or not. In this series of cases the first serum available was of low potency,[†] so that weak and moderately positive tests on retained Rh-positive cells resulted in cases being classified as Rh-positive. With strong anti-Rh serum typical pictures of good clumps of Rh-positive cells surrounded by unagglutinated Rh-negative cells will usually indicate the true situation. In cases with retained Rh-positive cells further and more serious sensitization should be avoided by the administration of only Rh-negative blood if more transfusions are necessary.

Aside from the possibility of immediate or remote reactions, the evidence in the cases described above indicates that the transfusion of Rh-positive blood to Rh-negative individuals is inefficient because of the rapid elimination of the transfused red cells in many cases.

Summary and Conclusions

In a series of wounded soldiers investigated after transfusion of whole blood there was evidence of abnormally rapid elimination of transfused Rh-positive red cells from the circulation of some Rh-negative recipients.

While demonstrable antibodies were found in only one case, it is quite possible that iso-immunization and latent sensitization occurred in others of this group.

The mechanism of rapid loss of Rh-positive red cells from Rh-negative recipients is obscure. Various extrinsic factors may have influenced this phenomenon in certain cases in this series, or some as yet undetected antibody may play a part in this haemolytic process. The possibility that an activated reticulo-endothelial system (or other cellular agency) may be involved in the rapid elimination of Rh-positive cells deserves further study.

From the practical standpoint, Rh-negative individuals should be transfused with Rh-negative blood. While Rh iso-immunization may

[†] This serum could not be refrigerated in transit, and lost its potency.

present few serious immediate reactions, any possibility of Rh sensitization should be avoided.

I am grateful for the unfailing kindness, assistance, and advice of Dr. R. R. Race and the late Dr. G. L. Taylor, of the Galton Laboratory Serum Unit, Department of Pathology, Cambridge University. Thanks are due also to the members of the staff and the laboratory personnel of the 163rd (U.S.) General Hospital for their co-operation and help in this study.

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ASYMPTOMATIC KALA-AZAR IN SOLDIERS FROM OVER-SEAS

BY

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The object of this note is to draw attention to the ease with which kala-azar may be overlooked in patients who make no spontaneous complaints. Two cases are described; both occurred in British troops who had served on the Mediterranean littoral. The first patient was seen in Italy and the second in England 20 months after his return from North Africa. Both, when pressed, admitted minor symptoms of several months' duration: these they had considered too trivial to warrant attendance on sick parade.

Classical kala-azar presents usually as a high swinging pyrexia, with periods of remission, and with marked toxæmia and sweating during the pyrexial periods. In both the cases reported here the fever was either slight or trivial, and toxæmia was absent.

Case I

Pte. H., aged 20. This man served in North Africa from Nov., 1942, to Nov., 1943. His disease probably originated in North Africa, for on arrival in Italy in Nov., 1942, he was stationed in the dock areas of Salerno and Naples, where kala-azar is not likely to be encountered. In May, 1944, he was ordered to report sick by his section sergeant, and was admitted to hospital. On direct questioning he admitted to a slow onset of general malaise, loss of weight, lassitude, and easy fatigability for the past 5 months. During this time he had continued to discharge his normal duties.

On examination he was thin and anaemic, and was found to be running an intermittent low-grade pyrexia up to 99° F. There were no abnormal signs in the heart or the lungs. The spleen was soft, was very difficult to feel, and was enlarged to three or four finger-breadths below the costal margin. He was seen by several medical officers and specialists, who expressed doubt as to the splenic enlargement owing to its extreme softness and the absence of a definite edge. It was not until three or four weeks after his admission that the spleen was unreservedly accepted as being enlarged. The liver was normal in size. On the basis of the presenting clinical findings, and in the absence of a definite splenic enlargement, he was at first thought to be suffering from pulmonary tuberculosis; this was excluded by radiography.

The results of investigations were as follows. Many malaria films were negative. May 13: R.B.C., 3,120,000 per c.mm.; Hb, 59%; W.B.C., 4,850 per c.mm. (polymorphs 34%, lymphocytes 53%, monocytes 9%, eosinophils 4%); B.S.R., 58 mm. in 1 hour. May 26: W.B.C., 2,160 per c.mm. (polymorphs 24%, monocytes 5%, lymphocytes 70%, eosinophils 1%). Formol-gel test, negative. June 9: At this point the splenic enlargement was accepted as genuine, and in view of the marked leucopenia and granulopenia a marrow puncture was performed. Leishman-Donovan bodies were found in moderate numbers in the bone marrow. An inguinal gland was punctured but showed no parasites in the stained film.

The patient was treated with stilbamidine (diamidinostilbene), and made a slow but uneventful recovery.

Case II

Gnr. B., aged 25. This soldier served in North Africa from Jan., 1943, to Jan., 1944, when he was invalided to the United Kingdom with a severe attack of diphtheritic polyneuritis. He recovered com-

pletely and was returned to duty, remaining in England until the onset of his present illness. In Sept., 1944, he was admitted to hospital with an anxiety state following the flying-bomb attacks, and says that he was given a full physical examination at this time. After his return to duty he continued his work for nearly a year, and in Sept., 1945, came up for routine medical examination before demobilization. He was found to have a very large spleen and was admitted to hospital for investigation.

On admission on Sept. 23 he made no spontaneous complaints, but on direct questioning admitted to some dyspnoea on exertion for about 12 months, and had noticed that his belly had been increasing in size for about 9 months. He was unaware of any pyrexia, but had had occasional shivering attacks. He did not believe that he had become thinner. He denied ever having suffered from malaria.

On examination he was pale and thin. His complexion was sallow, and there was a slight brownish pigmentation of the anterior axillary folds. His temperature chart showed a nightly rise to 100°, with an occasional double crisis during a 24-hour period. There was a mild tachycardia, the pulse rate varying between 90 and 100. Sweating was not marked. The tongue was clean and moist. The chest showed dullness and diminished air entry at the left base owing to the enormous size of his spleen, but the lungs and heart were otherwise clear. The abdomen was tense and showed on its surface a number of dilated veins. The spleen extended well below the umbilicus and just crossed the midline. It was hard but not tender. The liver was almost certainly enlarged to two to three finger-breadths below the costal margin, but it was covered with a resonant layer of colon, and neither an edge could be felt nor dullness to percussion be demonstrated. The lymph glands were normal in size and consistency.

Investigations resulted as follows: R.B.C., 3,120,000 per c.mm.; Hb, 55%; W.B.C., 1,400 per c.mm. (polymorphs 38%, monocytes 5%, lymphocytes 57%). Marrow puncture showed many Leishman-Donovan bodies. Ring forms of *Plasmodium vivax* were also seen. Gland puncture: Leishman-Donovan bodies were also easily found in smears made from the juice extracted by puncture from an apparently normal inguinal lymph gland. Formol-gel test, strongly positive.

The patient is at present being treated with stilbamidine.

Discussion

Though a rare complaint in British troops even on the shores of the Mediterranean, where the disease is endemic, kala-azar will certainly be seen for a time in England in troops who have returned from over-seas. In such persons it is not likely to occur very commonly, but it may well compare with the rarer blood diseases in frequency of appearance. If the mind of the physician is not attuned to the possibility of its appearance kala-azar may well be wrongly labelled and treatment withheld, with serious results.

The differential diagnosis in the slightly atypical forms recorded here should not be difficult. At first sight they may resemble pleurisy with effusion or phthisis. When the spleen is found, chronic malaria offers itself as an obvious diagnosis. When blood films are negative the chronic leukaemias come into question. The characteristic leucopenia may then suggest an "aleukaemic phase" in a myelocytic leukaemia, or perhaps some disease allied to the ill-defined Banti syndromes and splenic anaemias. In such cases sternal-marrow films are almost certain to be made; but the diagnosis may be missed even then, for the Leishman-Donovan bodies may be overlooked if the pathologist is not on the watch for them and has not been specially briefed by the clinician. They are often scanty in the bone marrow and may require a long search. If the bone-marrow films are negative it is always worth while to examine the juice obtained from the inguinal glands by puncture with a hypodermic needle. This should be done even if the glands are normal in size.

Summary

Two well-advanced cases of kala-azar with only slight fever and toxæmia are described.

The differential diagnosis, in relation to practical medicine in England, has been very briefly discussed.

It is stressed that further cases will occasionally be seen. A special watch should be kept for them.

S. Ben-Asher (*Ann. intern. Med.*, 1945, **23**, 431) records the case of a woman, aged 43, with mitral stenosis and auricular fibrillation in which an embolus in the right kidney resulted in rise of the arterial pressure. The blood pressure returned to normal with the disappearance of symptoms of renal infarction.