also states that the relative frequency of gene R'' to gene R_2 as compared with R' to R_1 indicates that his mythical gene locus c lies between d and e . However, these statements are based entirely on the findings in England and disregard the findings in other countries. In America, for example, the relative frequency of R' to R'' is reversed, while in negroes the frequency of r^o (42.1%) is quite different from the sum of R' (2.7%) and R'' (none found).¹⁷ As a result Fisher's theory leads to a contradiction. In my opinion a much more plausible explanation is that the genes r^o , R' , and R'' arose by mutation. Thus the high frequency of gene r^o and of the intermediate R genes, as well as the intermediate gene $A_{1, 2}$, suggests that the peculiar distribution among negroes is due to a higher mutation rate in this race.

The argument that the discovery of the so-called c^w factor¹⁸ supports Fisher's theory does not seem correct, because this factor appears to have the characteristic predicted by me^{15} for factor Rh''' .

In this connexion I should like to point to a recent attempt¹⁹ to develop an elaborate theory of inheritance of the four blood groups by means of four gene couplets. On the basis of this theory, Myslivec derives formulae for the gene frequencies involving the square root of the difference $0-2$ AB. Since populations exist in this world where the frequency of group O is less than twice the frequency of group AB, the theory leads to an obvious contradiction.

In conclusion I should like to take this opportunity to answer the rather academic attack²⁰ on my use²¹ of the term "conglutination" in connexion with the Rh antibody reactions. Recent findings²² have all sought to justify my use of this term. The term "co-agglutination," used by other workers, applies to a phenomenon obviously identical with mixed agglutination, and one would expect this to occur in conglutination where the second stage is non-specific.²³ In agglutination, involving bivalent antibodies, where the second stage is specific, co-agglutination or mixed agglutination does not occur. My new ideas and findings concerning univalent (blocking antibodies or glutinins) and bivalent antibodies (agglutinins) have led me to the theory^{24, 25} that erythroblastosis foetalis actually comprises three separate entities instead of a single disease. According to my proposed nomenclature, these three entities are as follows: (1) Congenital haemolytic disease (with anaemia and hydrops), usually due to univalent Rh antibodies; (2) icterus gravis with kernicterus, usually due to bivalent Rh antibodies; and (3) icterus praecox (formerly confused with "physiologic" icterus, usually due to A-B sensitization. Further details will be given in papers now in the press and in preparation.

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From a world starved of all types of scientific and surgical equipment by six years of war, inquiries and orders are pouring into Britain for products in this field. To cope with this new demand, a group of leading manufacturers announce the formation of a new company, "SCIEX" (Scientific Exports (Great Britain) Ltd.), with offices at Buckingham House, Buckingham Street, London, W.C.2. Their plans include group selling in overseas markets by a wide development of the existing agency organization of individual members. New agents are also being appointed in territories where member companies had no previous export business.

THE Rh FACTOR AND HEPATOMEGALY AND SPLENOMEGALY IN CHILDREN AND ADOLESCENTS

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Infants affected with congenital haemolytic disease may sustain damage to liver or spleen, or both, as manifested by jaundice and hepatomegaly, or splenomegaly, during early infancy. It seemed, therefore, of interest to apply Rh factor investigations in families in which there were children or adolescents suffering from enlargement of liver or spleen, on the ground that the aforementioned damage might become manifest in childhood by enlargement of these organs. Accordingly, three families were investigated and data concerning them are given below. The affected children were referred for diagnosis to the paediatric department of the Cardiff Royal Infirmary. Rh factor investigations were carried out in the laboratory of the Welsh Regional Transfusion Service. In two of the three families rhesus-immunization of the mothers was proved, but in the third family this was definitely excluded. The method of proof was the biological test, as the usual *in vitro* tests failed to reveal Rh iso-antibodies in the sera of the mothers. These cases are of much interest, since the affected children had enlargement of the liver or spleen, and such diagnoses as von Gierke's disease (glycogen disease), Gaucher's splenomegaly, Banti's disease, familial cirrhosis of the liver, etc., had been considered from time to time. Before giving an account of the families and investigations, the biological test may here be reviewed.

Oehlecker (1921, 1924) originally devised the biological test to determine whether blood was compatible for transfusion. The test was the rapid intravenous injection of 20 ml. of the proposed donor's blood, and a further similar quantity if after an interval of several minutes there were no symptoms. If the second injection caused no symptoms the donor's blood was regarded as compatible and its transfusion proceeded with. The donor's blood was considered incompatible if symptoms such as tingling, chill, nausea, etc., were caused by the small trial injection. This test, however, is not entirely reliable, because transfusion of incompatible blood may cause no symptoms (Drummond, 1944). Oehlecker's test, which in effect is simply a trial transfusion of blood, was subsequently adapted by Wiener, Wexler, and Gamrin (1944) for determining whether a particular individual had been previously sensitized to the Rh factor. It was known that *in vitro* tests might fail to demonstrate Rh agglutinins or blocking antibody in the sera of a small percentage of Rh-negative mothers who had had erythroblastotic infants, and in such cases the biological test was successfully used to demonstrate previous rhesus-immunization of the mother.

The biological test (see Wiener, Silverman, and Aronson, 1942) is carried out as follows: The mother to be investigated for previous immunization to the Rh factor is transfused with 50 ml. of fresh blood from a known Rh-positive donor. The ideal donor is the husband. The red cells of the donor must be compatible in the A-B-O system with the recipient's serum. Immediately before transfusion a 10-ml. sample of venous blood is taken from the mother with a clean, dry sterile syringe and divided between two tubes, one dry and the other containing 1 ml. of sodium citrate solution. One hour after the transfusion a further 10 ml. of the recipient's venous blood is taken and treated in the same way as the first sample. The serum and plasma of the pre- and post-transfusion samples of the recipient's blood are then compared. If the donor's blood is compatible the pre- and post-transfusion samples of the recipient's serum and plasma will be of the same colour—usually a pale straw colour. Hyperbilirubinaemia in the post-transfusion serum sample, along with its absence from the pre-transfusion serum sample, indicates that the donor's red cells are incompatible and have undergone rapid destruction in the recipient. When the biological test is positive the transfusion is usually, but not always, complicated by a febrile reaction with chill, rigor, etc. A cross-matching test by a reliable tube technique before transfusion should,

of course, have shown the donor's red cells to be compatible with the recipient's serum; it is a positive biological test which demonstrates the incompatibility. It has been pointed out by Mollison (1943) that even though an Rh-negative woman has, many years previously, been immunized to the Rh factor by bearing an Rh-positive foetus, and the Rh antibodies have long since disappeared from her serum, yet transfusion of Rh-positive blood will rapidly evoke the Rh agglutinins, with consequent haemolysis of the donor's blood.

Case Report: First Family

Mrs. M., aged 47, had never had a blood transfusion and has had the following pregnancies:—First: 1923, male (Ll.); no history of jaundice—further data below. Second: 1925, female (Els.); normal. Third: 1927, male (Rbt.); no history of jaundice—further data below. Fourth: 1930, male (On.); normal. Fifth: 1933, female (Jy.); slight jaundice soon after birth, which persisted three days. Sixth: 1936, male (Gd.); died intensely jaundiced, aged 3 days. Seventh: 1939, male (Gl.); apparently normal. Eighth: 1940, female (Jan.); moderately jaundiced for a few days after birth; now apparently normal.

The first child (Ll.) weighed 9 lb. (4.08 kg.) at birth. When 3 years old he underwent inguinal herniotomy. His abdomen has always been large, and he did not walk until 3 years old. He was stated to be a trifle slow mentally, though he attended an ordinary school. He occasionally had loose pale stools. The third child (Rbt.) weighed 8½ lb. (3.85 kg.) at birth and had a similar history, including operation for hernia. Neither of these boys was ever jaundiced.

Both boys were referred for diagnosis in December, 1936, because of much-enlarged bellies since birth. The first (Ll.) was at this time 13 years old and was slightly stunted in growth: height 4 ft. 3½ in. (131 cm.) (normal for age, 4 ft. 10 in. or 147 cm.); weight, 5 st. 5 lb. (34 kg.) (normal for age, 6 st. or 38 kg.). When standing his abdomen was protuberant and there was slight genu valgum (Fig. 1). Some telangiectases were present on the limbs and face.

FIG. 1.—First family. Left to right: First child (Ll.) and third child (Rbt.), with liver areas mapped out. The boy standing on the right is the fourth child (On.), who was apparently normal.

The liver was grossly enlarged, its lower border being down to the level of the umbilicus, and smooth and firm on palpation. Spleen not palpable. Mentally he seemed normal, but the I.Q. was not estimated. The condition of the third child (Rbt.), then aged 9 years, was similar to that of Ll. but his liver was much larger, for its lower border was felt well below the umbilicus. The spleen was just palpable, and there was a well-marked genu valgum (Fig. 1). There was some degree of infantilism, his height being 3 ft. 7½ in. (111 cm.) (normal for age, 4 ft. 2 in. or 127 cm.) and weight 3 st. 10 lb. (23.6 kg.) (normal for age, 4 st. 2 lb. or 26.3 kg.). The parents were healthy, and the second, fourth, and fifth children (then aged respectively 11; 7, and 3 years) were normal, while the remaining sixth child had died, intensely jaundiced, when aged 3 days.

The Wassermann reactions of the parents and Ll. and Rbt. were negative. In both boys the red cells showed slight decreased fragility. The blood-sugar curve was normal in the case of Ll., but there was some delay in return to normal in the case of Rbt. A laevulose-tolerance test on Ll. was normal. In both boys the van den Bergh test was negative. Each boy had a Hb of 74%; neither had a leucopenia. At this time (December, 1936) a diagnosis of familial cirrhosis of the liver was made. The other children were reported as normal at this time, and had no liver or splenic enlargement. Nothing further was heard of these two boys until 1945, when it was decided to apply Rh factor investigations. Ll. was examined in January, 1946; he was then of about average height, and his liver and spleen were no longer palpable. It will be noted that since the examination of the first and third children, in 1936, two further children (seventh and eighth, both Rh-positive) were born, the last of which had jaundice and recovered, whilst the other was apparently normal.

Rh Factor Investigations (July, 1945).—The mother was group O (IV) Rh-negative, and her serum contained neither Rh agglutinins nor blocking antibody. The Diamond-Abelson (1945) slide test was negative. Wiener's (1945) so-called conglutination test was difficult to interpret because of rouleaux formation, but the reaction was in all probability negative. The father was group B Rh+ (heterozygous). The children's groups were: Ll., O Rh+; Els., B Rh+; Rbt., B Rh+; On., B Rh+; Jy., O Rh-negative; Gl., O Rh+; Jan., O Rh+. On Jan. 14, 1946, the biological test was performed. The donor was Ll., the first child (group O Rh+). The father could not be the donor, as he was group B. The donor's red cells were compatible with the recipient's serum, and vice versa. Fifty ml. of the donor's blood was taken into 350 ml. of sodium citrate solution and at once transfused. The recipient, of phlegmatic temperament, became restless, anxious, and flushed when about half the blood had been transfused. Transfusion was completed in 30 minutes. The temperature was normal throughout, but an hour after transfusion it rose to 100.2° F. (37.9° C.). Thirty minutes after transfusion the recipient had a moderately severe rigor lasting 20 minutes. After five hours the patient's condition was normal. The pre-transfusion samples of the recipient's serum were a pale straw colour, but post-transfusion samples taken 1½ hours and 2½ hours after transfusion were deeply icteric. A catheter sample of urine collected an hour after transfusion was orange-coloured; there were no casts. A sample of the recipient's serum was collected on the 18th day after transfusion; it contained Rh agglutinins (anti-D) of titre 1/16. The Coombs *et al.* (1945a, 1945b) anti-human-globulin test, using a pre-transfusion sample of the mother's serum, gave a negative reaction.

Case Report: Second Family

Mrs. I., aged 42, had never had a blood transfusion and has had the following pregnancies:—First: 1934, male (Wm.); normal. Second: 1937, male; died of pertussis aged 5 weeks. Third: 1939, female (Pt.); full-term and normal at birth; no jaundice—further data below. Fourth: 1940, female; stillbirth at full term, but foetus was alive one week before delivery. Fifth: 1941, female; stillbirth at 28th week. Sixth: 1943, male; stillbirth at 26th week.

The third child (Pt.) was first seen in June, 1945, because of herpes zoster. Two years previously a school medical officer noted that she had an enlarged spleen. Apparently the child was not jaundiced at birth, nor at any time subsequently, but her abdomen had always been noticeably enlarged. Examination (in 1945) revealed a considerably enlarged spleen extending almost to the umbilicus. The liver also was enlarged, its lower border being 3 in. (7.6 cm.) below the costal margin (Fig. 2). The patient was a trifle undersized for her age. The haemoglobin was 76% and leucocytes 6,400. Fragility of red cells, normal. A tentative diagnosis of Banti's syndrome with cirrhosis of the liver was made. In January, 1946, the condition of the spleen and liver was unaltered. The parents are healthy, and the mother's Wassermann reaction is negative.

Rh Factor Investigations (July, 1945).—Mother, group O Rh-negative; no Rh agglutinins or blocking antibody detected in her serum. The third child (Pt.) was O Rh+, and her cells were not agglutinated by her mother's serum. Husband, O Rh+. First child (Wm.), O Rh+. The Diamond-Abelson (1945) slide test was negative, but the so-called conglutination test of Wiener (1945) gave a positive reaction.

It was decided to obtain more certain evidence of rhesus-immunization of the mother by applying the biological test. The husband of Mrs. I. was not available (being abroad), so a group O R₁R₂ donor was used (in case the mother was immunized to either the R₁ or the R₂ antigen). On Dec. 11, 1945, 50 ml. of the donor's blood was taken into 350 ml. of sodium citrate solution and at once transfused into the mother. Preliminary cross-matching tests showed that the donor's cells were compatible with the recipient's serum. Transfusion was given slowly over one hour because the recipient soon became restless and alarmed and experienced tingling sensations, throbbing in the head, nausea, vomiting, and pain in the lumbar

region. The reaction was fairly severe, and included rigor and rise of temperature from normal to 102° F. (38.95° C.). Samples of the recipient's serum collected one hour and four hours after the transfusion were markedly icteric compared with the pre-transfusion serum sample. A catheter sample of urine taken before transfusion was water-clear, but a similar sample taken one hour after transfusion was orange-amber in colour; no urinary casts were found.

FIG. 2.—Second family. Third child (Pt.). Area of liver and spleen mapped out.

On the ninth day after the biological test strong Rh agglutinins of titre 1/64 (anti-D) were found in the recipient's serum. Finally, in January, 1946, the Coombs anti-human-globulin test was carried out on a pre-transfusion sample of the recipient's serum, and a positive reaction was obtained.

Case Report : Third Family

Mrs. L., aged 42, had never had a blood transfusion. She has a cleft palate, and has had the following pregnancies:—First: 1930, female, one month premature; moderately jaundiced for two weeks and died of pneumonia aged 16 months. Second: 1932; abortion at seventh week. Third: 1933, twins; one was of undetermined sex and died soon after birth; the other, a female, had convulsions and died, intensely jaundiced, on the eighth day. Fourth: 1935, male (M.); two weeks premature; never jaundiced; surviving and normal. Fifth: 1938, female (J.); became moderately jaundiced a day or two after birth, and jaundice persisted three months. Enlargement of liver detected when the child was a month old.

The fifth child (J.) has been under observation intermittently since infancy for hepatomegaly and splenomegaly. In December, 1945, her abdomen was very protuberant. The spleen was much enlarged and extended 3 in. (7.6 cm.) medially from beneath the left costal margin. The lower border of the liver was palpable two fingerbreadths below the right costal margin. In recent months epistaxes have occurred. A blood examination revealed slight secondary anaemia and a leucopenia. The child was intelligent and of about normal size. The mother's Wassermann reaction is negative. The clinical diagnosis was considered to be Banti's disease or splenic anaemia.

Rh Factor Investigations.—Mother, A, Rh+ (R,R^o), and no irregular antibodies detected in her serum. Husband, O Rh+ (R,r). M. (fourth child), A, Rh+ (R^or). J. (fifth child), A, Rh+ (R,R^o). Wiener's conglutination test was negative. The Coombs anti-human-globulin test, using the red cells of the children, was negative. The biological test, using the husband as donor, gave a negative reaction. Subsequent to the biological test no irregular agglutinins appeared in the mother's serum. There was therefore no evidence of iso-immunization of the mother to an antigen in the red cells of her foetuses.

Discussion

In some infants affected with congenital haemolytic disease, when Rh agglutinins or an incomplete antibody has been demonstrated in the maternal serum, we have observed liver or

splenic enlargement, or both, in addition to jaundice. Occasionally such enlargement of liver or spleen persisted for several weeks after jaundice had disappeared. Other investigators—for instance, Wiener and Wexler (1943), Wiener (1944), Wiener, Wexler, and Gamrin (1944), and Langley and Stratton (1944)—have also observed jaundice, hepatomegaly, and splenomegaly in newborn infants affected with haemolytic disease. The relationship between neonatal jaundice and liver damage and the Rh factor has recently been dealt with by Skelton and Tovey (1945).

There are not, apparently, any reports on Rh factor investigations in families in which there were older children or adolescents with liver or splenic enlargement. Since previous rhesus-immunization of the mothers was clearly proved in the first and second families in this report, it is possible that the hepatomegaly and splenomegaly in these children is a late manifestation of damage caused by the noxious action of Rh iso-antibodies. In this connexion it is to be noted that Rh group-specific substances have been demonstrated in the liver, spleen, kidney, etc., by Boorman and Dodd (1943). Cappell (1944) states: "The severity of foetal liver damage in some cases of haemolytic disease is an indication that harmful effects are by no means confined to the blood and haemopoietic organs."

If damage by Rh iso-antibodies was the cause of the liver and splenic enlargement in the affected children of the first and second families, it then becomes intriguing to know why, in the first family, the second, fourth, and seventh children, who were all Rh-positive, were not affected with jaundice, hepatomegaly, or splenomegaly, whereas the sixth infant died jaundiced and the eighth had a moderate degree of jaundice but recovered spontaneously. The Rh-negative child in family No. 1 has always been normal. That the first child in family No. 2 was not affected, although Rh-positive, is understandable, for it is well known that in haemolytic disease the firstborn is frequently unaffected. However, since rhesus-immunization of the mothers was proved in the first two families, it seems likely that congenital haemolytic disease was the cause of the liver and splenic enlargement. The cases of the children affected with splenomegaly and hepatomegaly in the first two families are unusual in that jaundice apparently never occurred. It is, however, well known that some infants affected with congenital haemolytic disease may be profoundly anaemic though never jaundiced. On the other hand, in the third family the affected child was jaundiced for several weeks, but the possibility of rhesus-immunization of the mother was definitely excluded.

Familial cirrhosis of the liver has long been recognized clinically, and our findings in the first family suggest that in such cases Rh factor investigations are essential in a search for the true cause. It would also seem, from our findings in the second and third families, that Rh factor investigations should be applied in cases diagnosed clinically as Banti's disease or splenic anaemia in children. Liver and splenic enlargement in children may, apparently, in some cases be attributable to congenital haemolytic disease, and, if this is so, Rh factor investigations are essential in the differential diagnosis of unexplained forms of hepatomegaly and splenomegaly occurring in children or adolescents.

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