A RABBIT SERUM CONTAINING A SPECIFIC AGGLUTININ FOR THE RED CELLS OF THE NEWBORN

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We have for some time, in collaboration with Dr. P. W. Hutton, of Mulago Hospital, Kampala, Uganda, been attempting to repeat the experiments of Schneider and Levin (1950) in which they obtained rabbit antisera giving specific agglutination of the erythrocytes of sickle-cell anaemia patients. These particular experiments have, so far, been inconclusive, but in view of the definite results reported below, which we have now obtained by the injection of foetal erythrocytes into rabbits, we hope to continue our investigation of the response to the injection of sickle-cell anaemia erythrocytes.

An outstanding finding reported by Schneider and Levin was that their antisera agglutinated the red cells of sickle-cell anaemia patients but not of sickle-cell trait carriers. One of the differences between these two is the presence in the former, but not in the latter, of an alkali-resistant haemoglobin, thought to be foetal haemoglobin (Singer, Chernoff, and Singer, 1951). It has now been shown that this alkali-resistant haemoglobin is antigenically indistinguishable from true foetal haemoglobin (Chernoff, 1953 ; Goodman and Campbell, 1953). Foetal haemoglobin is present in nearly all types of haemolytic anaemia, and its clinical importance is shown by Table I, reproduced from Itano (1953). This table was published before Chernoff or Goodman and Campbell reported the presence of traces of foetal haemoglobin even in normal adults and sickle-cell trait carriers.

TABLE I.—*Distribution of Different Haemoglobins (From Itano, 1953)*

Condition	Haemoglobin				
	a (Normal Adult)	b (Sickle Cell)	c	d	f (Normal Foetal)
Normal adult	+	−	−	−	−
,, newborn	+	−	−	−	+
Sickle-cell trait	+	+	−	−	−
,, ,, anaemia	−	+	−	−	+
Haemoglobin-c trait	+	−	+	−	−
Sickle-cell haemoglobin-c disease	−	+	+	−	±
Haemoglobin-d trait	+	−	−	+	−
Sickle-cell haemoglobin-d disease	−	+	−	+	+
Thalassaemia minor	+	−	−	−	±
,, major	+	−	−	−	+
Sickle-cell thalassaemia disease	+	+	−	−	+
Some acquired haemolytic anaemias	+	−	−	−	+

We are not in a position to assert that the serum of Schneider and Levin, which acts on the cells of sickle-cell anaemia but not on those of the sickle-cell trait, agglutinated specifically cells containing foetal haemoglobin, but a consideration of this possibility, and the fact that the one feature common to nearly all haemolytic anaemias is the presence of foetal haemoglobin in the red cells in appreciable quantity, led us to attempt to prepare a specific agglutinin for human foetal red cells. We considered that such an agglutinin might be of value in the investigation of haemolytic anaemias both of infants and of adults.

Preparation of Sera

Unclotted blood was obtained by syringe from the placental portion of the cord when on delivery the circulation between placenta and the newborn was interrupted. In most cases this was then allowed to clot and red cells were subsequently obtained by breaking up the clot with a glass rod. In some cases the blood was heparinized. In each case the red cells were washed five times with an ice-cold solution of 4% glucose and 0.18% sodium chloride. They were then suspended in the glucose-saline solution so that 1 ml. of the final suspension contained 0.3 ml. of packed cells. The suspension was stored at 4° C. in separate lots of 3–4 ml. each. Three adult rabbits were each inoculated intravenously with 0.8 ml. of the suspension on each of five consecutive days ; this treatment was repeated once every four to six weeks, and ten days after the last injection the rabbits were bled ; the blood was allowed to clot and the serum was removed. After inactivation at 55° C. for 30 minutes the serum was frozen and kept in that state until it was used for absorption and agglutination experiments. The sera of rabbits I, II, and III showed only little agglutinating power after the third course ; after the fourth course, however, there was an impressive rise in titre in the serum of rabbit II, and even more in that of rabbit III, but not in that of rabbit I ; rabbit I showed a rise of titre, however, after the fifth course. This communication deals only with the sera of rabbits II and III obtained after the fourth course.

As the unabsorbed sera showed virtually no anti-M agglutinin they were absorbed with adult human red cells of group A_1N. Serum II was diluted twenty times with isotonic saline and the diluted serum was absorbed with three-quarters of its volume of packed A_1N cells ; serum III was diluted ten times and absorbed with an equal volume of the cells. After this treatment adult human red cells were only slightly agglutinated (see Table II), which was attributed to the persistence of anti-human species agglutinin.

The absorbed sera were titrated against cord and infant blood cells, together with normal adult controls, so far as possible of the same ABO and MN groups. Other adult specimens were tested in order to eliminate possible effects of blood-group systems other than ABO and MN (Table II). Table II shows ABO, MN, and, in view of their irregular behaviour in infancy, also the Lewis blood groups for each individual. The rhesus groups for adults and infants respectively were as follows :—CCDee : 3, 3 ; CcDee : 5, 7 ; CcDEe : 3, 1 ; C^wcDEe : 1, 0 ; ccDEE : 0, 2 ; ccDee : 2, 0 ; ccddee : 4, 4. Ten of the adults and seven of the infants were positive for S. The titrations for P for adults and infants respectively were as follows :—+++ : 6, 2 ; ++ : 3, 1 ; + : 4, 9 ; − : 7, 8. There was one Lu^a positive in each group. Four of the infants were not tested for Kell and three not for Fy^a ; the results for adults and infants were :—Kell positive : 2, 1 ; Fy^a positive : 15, 9.

Titration Techniques

Saline Titration.—Serial twofold dilutions of the sera were carried out with isotonic saline, and an equal volume of 3 to 4% cell suspension in saline was added to the fluid in each tube. The tubes were incubated at 37° C. for two hours, and

Table II.—*Albumin Titration of Sera II and III against Adult, Cord, and Infant Cells. The Titres refer to the Dilution of the Serum before the Addition of an Equal Volume of Red-cell Suspension.*

No.		ABO	MN	Lea	Leb	Serum II Dilutions							Serum III Dilutions								
						1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120
1	Normal adult	O	M	−	+	4	2	1					3	1								
2	,, ,,	O	M	−	+	4	1						4	1								
3	,, ,,	O	MN	−	+	1	0						0	0								
4	,, ,,	O	MN	+	−	1	0						0	0								
5	,, ,,	O	MN	+	−	2	2	1					2	2	1							
6	,, ,,	O	MN	−	+	2	1						2	1	1							
7	,, ,,	O	MN	−	+	4	1						1	0								
8	,, ,,	O	N	+	−	2	1						1	0								
9	,, ,,	O	N	−	+	4	2	1					4	1								
10	,, ,,	O	N	−	+	2	1						2	2	1							
11	,, ,,	O	N	−	+	3	1						1	0								
12	,, ,,	A_1	M	−	+	0	0						0	0								
13	,, ,,	A_1	M	−	+	2	1						1	0								
14	,, ,,	A_1	MN	−	+	2	1						1	0								
15	,, ,,	A_1	MN	−	+	0	0						0	0								
16	,, ,,	A_1	N	−	+	2	2	1					2	2	1							
17	,, ,,	A_1	N	+	−	3	1						1	0	0							
18	,, ,,	A_2	M	−	+	0	0						1	0								
19	,, ,,	A_2	MN	−	+	1	1						0	0								
20	,, ,,	B	MN	−	+	2	0						1	0								
21	7 days ..	O	M	+	−	5	5	4	4	1			5	5	5	5	5	5	4	4	2	1
22	3 ,, ..	O	MN	+	+	5	5	4	4	2	1		5	5	5	5	5	5	4	4	2	1
23	Cord	O	MN	−	−	5	5	4	4	2	1		5	5	5	5	5	5	4	4	2	1
24	24 days ..	O	MN	+	−	5	5	4	4	2	1		5	5	5	5	5	5	4	3	1	
25	Cord	O	MN	−	−	5	5	4	2	1			5	5	5	5	5	5	4	4	2	1
26	12½ months ..	O	MN	−	−	3	1						3	1								
27	Cord	O	MN	−	−	5	4	4	4	2	1		5	5	5	5	5	5	4	2	1	
28	,, ...	O	N	+	−	5	4	4	4	2	1		5	5	5	5	5	4	3	2	1	
29	,, ...	O	N	−	−	5	5	4	2	1			5	5	5	5	5	4	4	2	1	
30	,, ...	O	N	−	−	5	5	5	4	2	1		5	5	5	5	5	4	2	0		
31	,, ...	O	N	+	−	5	5	4	2	1			5	5	5	5	4	4	2	1		
32	20 days ..	A_1	M	+	−	5	5	4	4	3	1		5	5	5	5	5	5	4	4	3	1
33	Cord	A_1	M	−	−	5	5	3	2	1			5	5	5	5	4	4	3	2	1	
34	4½ months ..	A_1	MN	−	−	4	1						4	1								
35	Cord	A_1	MN	−	−	5	5	4	3	1			5	5	5	5	4	4	3	2	1	
36	9 days ..	A_1	MN	+	−	5	5	4	4	2	1		5	5	5	5	5	5	4	4	2	1
37	7 ,, ..	A_1	MN	+	−	5	5	4	4	2	1		5	5	5	5	5	5	4	4	2	1
38	Cord	A_2	MN	−	−	5	4	4	4	2	1		5	5	5	5	5	5	4	2	1	
39	6 days ..	A_2	N	+	−	5	5	4	4	4	2	1	5	5	5	5	5	5	4	4	2	1
40	5 months ..	B	N	−	−	1	0						0	0								

0=All cells separate and evenly distributed. 1=A definite but weak reaction in which there is a uniform distribution of very small clumps of 4–6 cells. 2=Clumps of 8–12 cells. 3=Not quite such big clumps as in 4; numerous unagglutinated cells. 4=Granularity just visible to the naked eye; very big clumps under the microscope; some unagglutinated cells. 5=Numerous clumps, visible to the naked eye.

the cells transferred to a slide and examined microscopically. All titres so observed were very low, and there were no constant differences between the cord and infant cells and the adult controls. The results are therefore not reported in detail.

Indirect Coombs Test.—The cells were suspended in saline and incubated with saline dilutions of sera II and III; the cells were then washed three times with saline and mixed on a tile with suitably diluted goat anti-rabbit-globulin serum. The cord and infant bloods gave somewhat higher titres than the adult controls, but the differences were not great and hence are not reported in detail.

Albumin Titration.—Suspension of red cells in albumin solutions is not commonly used in agglutination experiments with animal sera; we decided to try this procedure, however, and found it to give satisfactory results. Serial two-fold dilutions of the absorbed rabbit serum were made with normal adult human AB serum, and to each volume of diluted serum was added an equal volume of a 5% suspension of red cells in 20% bovine albumin. Incubation was carried out for two hours at 37° C., and results were read microscopically after transfer to slides. This method showed considerable and systematic differences between the cord and infant cells on the one hand, and the adult control cells on the other. The results are shown in Table II.

TABLE III

Serum	Ratio Serum: Packed Cells	Absorption
II	1:0·75	Not detectable
II	1:1	Partial
II	1:1·25*	Complete
III	1:1	Not detectable
III	1:2	Partial
III	1:2·5†	Complete

* Absorption with 1 volume of cells, followed by 1/4 volume.
† Absorption with 2 volumes of cells, followed by 1/2 volume.

Absorption.—In view of the possibility that the cord blood cells were clumping because of something other than the presence of a specific antigen, possibly a major protein constituent of the serum, absorption experiments were carried out, using the red cells of cord blood 33 for the purpose. Serum II was diluted twenty times and serum III ten times with saline, and they were absorbed at 4° C. overnight, with varying volumes of packed cells, then tested for agglutinating power against fresh cells of the same type. The results are shown in Table III.

Discussion

Only in the albumin titrations were fully conclusive results obtained. The red cells from ten cord bloods and from seven infants below the age of 1 month were strongly agglutinated, while there was only slight agglutination, or none, of the red cells of 20 healthy adults and of three infants aged 4½, 5, and 12½ months. The agglutinin responsible was absorbed out by cord blood cells. The blood groups of the agglutinated bloods and of the controls are such that none of the known blood-group antigens or any combination of these could be responsible for the observed agglutinations. The Lewis system deserves special attention in this connexion, since there tend to be too few Le^b positives in the newborn and too many Le^a positives slightly later, as compared with adult blood groups. An inspection of the Lewis groups of the bloods (see Table II) shows, however, that this system is not responsible for the differences in agglutinability.

Agglutination is generally thought to be associated with the composition of the red-cell envelope and not of its contents, yet our findings suggest that the presence or absence of foetal haemoglobin may have been the determining factor in the agglutination and that the abnormal haemoglobin is associated with a similar abnormality of the lipo-protein of the cell envelope. Since our tests were completed the papers of Chernoff and of Goodman and Campbell have

reached us, reporting the preparation of sera which precipitate foetal haemoglobin specifically from aqueous solutions The relation of these precipitins to our agglutinin remains to be determined. Chernoff reports that in one normal pregnant woman 2% of the total haemoglobin was present in the foetal form, whereas in twelve others the percentage was below 0.85, the upper limit in normal adults being 1.3. Since we have shown cord red cells to produce agglutinin in rabbits and the American authors have demonstrated foetal haemoglobin to be antigenic, it will be important to consider the possible consequences of transplacental introduction of such materials into the circulation of human mothers.

Summary

By the injection into rabbits of human cord blood cells, an antiserum has been prepared which agglutinates specifically the red cells of cord blood and of newborn infants.

We are grateful to Dr. R. R. A. Coombs for the supply of goat anti-rabbit-globulin serum and to the medical and nursing staff of the Department for Midwifery and Diseases of Women, St. Bartholomew's Hospital, for making available blood samples and helping in their collection. One of us (H. L.) thanks the Medical Research Council for a grant towards expenses.

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ALCOHOL-INDUCED PAIN IN HODGKIN'S DISEASE

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Alcohol, when taken by patients with Hodgkin's disease, may in some cases give rise to severe pain. When present this symptom is of considerable clinical value—by focusing attention to areas of active disease, and by serving as a reliable guide when judging response to treatment. No mention is made of this curious intolerance to alcohol in any of the classical textbooks or monographs dealing with Hodgkin's disease, and no case report describing this symptom seems to have been published in this country so far. Only two references to this subject could be traced in the medical literature (Hoster, 1950; Verbeeten, 1952).* Hoster, in America, referring briefly to this phenomenon in one of his articles on Hodgkin's disease, states that in a number of his cases pain occurred or, if already present, was increased at the site of Hodgkin's deposits shortly after intake of alcohol-containing drinks, and that such pain was frequently replaced by localized anaesthesia if adequate amounts of alcohol were taken. Verbeeten, in Holland, quotes case histories of four patients with Hodgkin's disease (three male and one female) who suffered pain of such intensity after drinking alcohol that they all became teetotal. After *x*-ray therapy one of these patients was able to take alcohol again without ill-effects; no information is given on the fate of the other three cases.

*Since the submittance of this paper a similar report, describing nine cases, has been published by two Danish authors (Bichel, J., and Bastrup-Madsen, P., *Lancet*, 1953, **1**, 764).

In view of the scanty published data on the subject, the following two cases are placed on record.

Case 1

A housewife aged 37 was referred to hospital in February, 1951, with painless enlargement, for the past two years, of glands at the base of the neck which had suddenly begun to increase in size very rapidly during the previous month.

On examination she was found to be in excellent general health. The glands in the left posterior triangle were enlarged and there was a fixed mass, the size of a billiard ball, in the right supraclavicular fossa. Section of cervical glands showed Hodgkin's disease. An *x*-ray film of the chest showed enlargement of the right paratracheal glands. A course of *x*-ray therapy to cervical and supraclavicular areas as well as to the mediastinum was given in March, 1951, with resulting complete regression of all enlarged

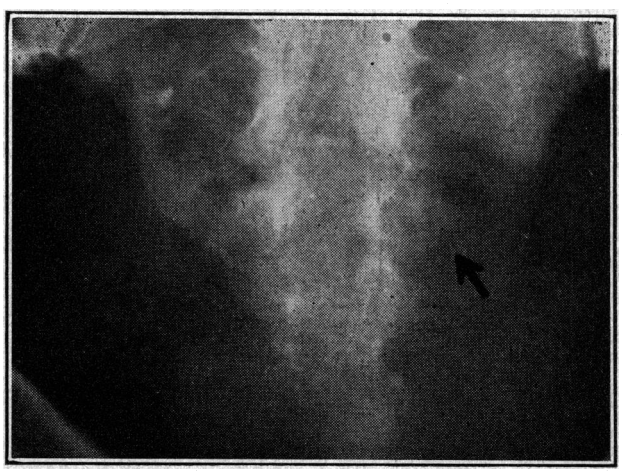

FIG. 1.—Case 1.

lymph nodes in the treated areas. She remained well until Christmas, 1951, when, on taking a small sherry, she experienced within a few minutes pain in the region of the coccyx on the left side which was so severe that she had to lie down. The acute pain passed within an hour or so, but the coccyx remained tender for three days. A week later, on New Year's Eve, she had only a few sips of sherry, which within a few minutes were again followed by most distressing pain in the region of the coccyx as before. When examined at the hospital on the following day there was marked tenderness on palpation in the left sacro-coccygeal region, but a radiograph of the coccyx at that time failed to reveal any definite bone disease. She was asked to have a sip of sherry in order to confirm the suspected relationship between pain and intake of alcohol. The resulting pain was so severe on this occasion that she decided to give up drinking alcohol altogether.

She remained well in herself throughout 1952, but continued to complain of slight tenderness on pressure in the left sacro-coccygeal region. She broke her 11 months' abstinence for the first time on December 9, 1952, when she had a cherry brandy. Within six minutes she was in terrible pain and "could not sit, stand, or walk." Finally, the severe pain was relieved by the patient lying in a hot bath. She was seen at the hospital within a week of this, and *x*-rayed again. Dr. Franklin reported as follows: "There is considerable bone destruction in the left sacro-coccygeal region, the appearances being consistent with the presence, in this region, of a lymphadenomatous deposit" (Fig. 1). A course of *x*-ray therapy to the affected left sacro-coccygeal region was started on December 31, and within two weeks the pain on taking alcohol was only very slight, and by the end of the three-weeks course she was able to take even large amounts of alcohol with impunity.