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THE UNIVERSAL DONOR WITH HIGH TITRE ISO-AGGLUTININS*

THE EFFECT OF ANTI-A ISO-AGGLUTININS ON RECIPIENTS OF GROUP A

BY

E. F. AUBERT, M.A., M.B., B.Chir. KATHLEEN E. BOORMAN
BARBARA E. DODD, B.Sc. AND J. F. LOUTIT, B.M., M.R.C.P.

The use of group O (universal donor) blood for recipients of other groups was first suggested by Ottenberg (1911). He argued that its anti-A and anti-B iso-agglutinins—theoretically incompatible for recipients whose cells possess the corresponding agglutinogens—would be so diluted by the recipient's plasma as to be rendered innocuous. In many places the use of group O blood for recipients of all groups has become established, but whether this practice is always advisable is still a matter for speculation.

Views in the Literature

Levine and Mabee (1923) issued a warning that group O blood might be dangerous for other groups if its iso-agglutinins were of high titre. This view has been stressed quite recently by Witebsky *et al.* (1940), who regard a considerable proportion of group O donors as potentially dangerous. Coca (Townsend and Coca, 1935; Coca, 1938) of the Transfusion Betterment Association in New York, holds that about 3% of universal donors have dangerously high titres and should not be used for the transfusion of patients of other groups. In Russia, Hesse (1935) believes that over 30% of group O donors have dangerously high titres; and a conference at Leningrad in 1935 condemned the use, even in wartime, of universal donor blood for recipients of other groups. Greval *et al.* (1941) in India, and Jakobowicz and Bryce (1941) in Australia, have also laid considerable emphasis on the dangers inherent in the "universal" use of group O blood.

On the other hand, a considerable body of opinion supports the belief that universal donor blood is safe for recipients of other groups. Brines (1930), in the U.S.A., cites 4,000 transfusions, mostly with universal donors, without a fatality. According to Riddell (1939), in France the Transfusion Sanguine d'Urgence of Paris, which supplies blood for over 6,000 transfusions a year, had not reported a single fatality up to 1939: group O donors formed almost the sole source of blood for this service. The general impression among workers in the transfusion services of this country is that the danger from the iso-agglutinins is negligible. This impression, based upon clinical experience, is supported by the following theoretical considerations:

(*a*) *Dilution.*—There is dilution of the transfused iso-agglutinins by the recipient's plasma (Ottenberg and later authors). In most cases, however, the titres of the iso-agglutinins are too high to be reduced to ineffective levels merely by the diluent action of the recipient's plasma.

(*b*) *Absorption of Iso-agglutinins by Tissues of Recipient.*—The appropriate agglutinogens A and B are present in the plasma as well as in the red cells of persons of groups A, B, and AB. Plasma containing these agglutinogens specifically inhibits *in vitro* the homologous iso-agglutinins (Schiff, 1924; Levinson and Cronheim, 1940; Della Vida and Dyke, 1941; Jakobowicz and Bryce, 1941; Aubert, Boorman, and Dodd, 1942). It seems probable that if these agglutinogens are present in the plasma of the recipient they will help to neutralize transfused anti-A or anti-B iso-agglutinins. Group-specific substances have also been demonstrated in many organs and tissue fluids of the body (Wiener, 1939). The cells of such organs specifically absorb iso-agglutinins *in vitro*, and it seems probable that absorption by tissue cells—for example, those lining the blood vessels—takes place *in vivo*. Erythrocytes are also able to absorb appreciable quantities of homologous iso-agglutinins without agglutination occurring.

(*c*) *Temperature.*—The titre of an iso-agglutinin is appreciably lower at body than at room temperature. If this fact is overlooked the *in vivo* activity of the agglutinin will tend to be overestimated. (The haemolytic activity of serum and plasma, however, increases with the temperature, and, although it is generally believed that the iso-agglutinins are responsible for haemolysis as well as for agglutination, the possibility that iso-haemolysins play a part in haemolytic reactions cannot in the present state of our knowledge be completely excluded.) Temperature may also influence iso-agglutinin inactivation. This is said to be more pronounced at body than at room temperature (Davis, 1941).

The remarkable disparity of views held about the "universal" donor has been due to lack of direct evidence. When a haemolytic reaction has followed the use of universal donor blood the iso-agglutinins cannot justifiably be blamed unless it can be shown: (i) that the transfused blood contained iso-agglutinins capable at body temperature and in considerable dilution of clumping the cells of the recipient; (ii) that the recipient's and not the donor's cells were destroyed. Examining the literature on haemolytic reactions which have followed the transfusion of group O blood into recipients of other groups, one finds that the first criterion is seldom and the second never satisfied. Of the 46 cases collected by Hesse (1935) only one was shown to have potent "incompatible" iso-agglutinins. In the few additional cases in which these have been demonstrated—such as the one reported by DeGowin (1937)—haemolysis of the donor's cells was not excluded.

The Present Investigation

Our objects were: (1) to confirm the range of agglutinin titres found by other workers in group O plasma; (2) to select blood from donors possessing high titre iso-agglutinins for transfusion (thus satisfying criterion (i) above); (3) to use plasma or serum rather than whole blood (thus avoiding the complicating factor of the donor's cells); (4) to control the transfusion of high titre group O plasma or serum with similar transfusions of low and moderate titre fluids. Recipients of group A were selected, as this is a common group and as the anti-A titre in group O plasma tends to be higher than the anti-B.

In assessing the results of these transfusions we observed in particular: the clinical reaction of the recipient; the effect on the recipient's erythrocytes; and the part played by the anti-agglutinin mechanisms in the recipient.

Technique of Iso-Agglutinin Titration

This was substantially the same as that described by Taylor and Ikin (1939). The titrations were carried out in 2 in. by 1/4 in. round-bottomed precipitin tubes. A 0.85% solution of

* A report to the Medical Research Council from the S.W. London Blood Supply Depot.

sodium chloride in distilled water was used as diluent. Equal volumes of serum, saline, and red cell suspension were measured by a Pasteur pipette marked to deliver approximately 0.04 c.cm. The A and B cells were obtained from the same two donors throughout. The cells were always less than 24 hours old when used, and a 2% suspension (in terms of whole blood and judged by eye) in a 3% sodium citrate solution was employed. The serum dilutions were doubled at each stage and an equal volume of red cell suspension was added to each tube, which was then shaken to make the suspension even. All titrations were done in duplicate.

The tubes were read after standing for two hours at room temperature. Readings were made after the cell deposit had been dispersed by tapping the tube sharply three or four times with the finger. No reaction was recorded as negative until it had been examined microscopically. For this purpose a drop of the well-mixed contents of the tube was spread evenly over a microscope slide by means of the stem of the Pasteur pipette used for its transfer. The end-point was taken as being that dilution in which clumps of 3 or 4 cells could be seen. This was accepted as evidence of agglutination only if the smear from the succeeding tube was clear. The titre is given by the reciprocal of the greatest dilution, calculated before the addition of the control cells, at which agglutination occurs (Wiener, 1939). Reading the end-point with the microscope was found to give more consistent results than reading with the naked eye. In addition it probably indicates agglutinin activity more accurately.

Room temperature varied between 50° and 70° F. The effect of this variation on iso-agglutinin titre is not great (Wiener), and did not seem enough to justify the difficulties of titrating at a constant temperature. If duplicate readings differed by one tube (this occurred about once in every 10 titrations) the lower reading was taken. If they differed by more than one tube the titration was repeated. The control A and B cells used throughout were of average sensitivity to the A and B iso-agglutinins.

Preparation of Serum and Plasma for Transfusion

The sera were prepared as follows. Blood was drawn from group O donors into dry, sterile one-pint M.R.C. transfusion bottles and allowed to clot. After 24 to 48 hours at room temperature, when the clot had retracted, the serum was pipetted off under aseptic conditions, pooled, and Seitz-filtered. About 200 c.cm. of serum could be obtained from each donor. Two or three donors were therefore required to make up the 400 to 500 c.cm. quantities that we chose as the unit of fluid to be transfused.

When plasma was used it was obtained from the Depot blood bank. 430 c.cm. of blood was taken into an anticoagulant solution containing 100 c.cm. of 3% sodium citrate and 10 c.cm. of 30% glucose. Sedimentation of the red cells was allowed to proceed for 4 to 6 days at 4° C. The supernatant plasma was then pipetted off under aseptic conditions. Two bottles of blood would provide 500 c.cm. of plasma. The bottles of plasma thus obtained were not Seitz-filtered, but were cultured before administration to check their sterility.

Samples of the transfused serum or plasma were titrated by the above method on the day of the transfusion, using both the usual standard A cells and the recipient's cells. In no case did the titre differ significantly between the two. The transfusions were administered by means of the M.R.C. " giving " apparatus. The fluids were not warmed beforehand.

Examination of Blood Samples from Recipient

Venous samples were withdrawn, using either a 16 S.W.G. needle without suction or a 20 S.W.G. needle and a dry, sterile syringe. If the venepuncture was cleanly made, the act of withdrawing the sample did not cause haemolysis detectable by our spectroscopic method. 10 mg. of Wintrobe's oxalate mixture was added to 5 c.cm. of blood as an anticoagulant.

The " inhibition index "—a crude measure of the amount of free agglutinogen in the recipient's plasma—was estimated by the method of Aubert, Boorman, and Dodd (1942). A glass standard Haldane haemoglobinometer was used for haemoglobin readings, which were made in duplicate by strong daylight (experimental error ±2%). Dilutions for red cell counts were made in a standard pipette. A Bürker haemocytometer was used, and at least 1,000 cells were counted (experimental error ±5%). The haematocrit figure was estimated by centrifuging blood in a Wintrobe tube at 2,500 revolutions a minute for one hour (experimental error ±1%). Bilirubin was estimated, where possible, in serum rather than plasma. A modified van den Bergh technique (Peters and van Slyke, 1932) with methyl red as standard was used (experimental error ±0.1%). Serum samples were examined with a pocket spectroscope in a 1-cm. layer for blood pigments. When positive they were forwarded to Mr. R. J. Bromfield for confirmation, and subsequent samples were examined by him for methaemalbumin and Schumm's " haematin." Potassium citrate was given by mouth to each recipient before transfusion, to alkalinize the urine.

Observations

Titrations were made of 250 unselected group O sera. Their anti-A and anti-B titres showed considerable variation (Table I). The anti-A titres were usually higher than the anti-B. The peaks of the two frequency curves occurred at 256 and 64 respectively. Both iso-agglutinins were present in every serum in this series. The highest anti-A titre recorded was 8,192, but titres of 16,384 have been found subsequently, and even higher ones are on record. The highest anti-B titre was 4,096. The lowest titres recorded were anti-A 16 and anti-B 2. High anti-A and anti-B titres usually occurred together, the former almost always being the higher.

TABLE I.—*Anti-A (α) and Anti-B (β) Titres of 250 Unselected Group O Donors*

Titre	Anti-A		Anti-B	
	No.	%	No.	%
1	—	—	—	—
2	—	—	1	0·4
4	—	—	3	1·2
8	—	—	13	5·2
16	5	2·0	25	10·0
32	11	4·4	51	20·4
64	17	6·8	56	22·4
128	51	20·4	44	17·6
256	65	26·0	34	13·6
512	63	25·2	17	6·8
1,024	23	9·2	3	1·2
2,048	8	3·2	2	0·8
4,096	5	2·0	1	0·4
8,192	2	0·8	—	—
	250	100·0	250	100·0

These results agree qualitatively with those obtained by previous workers (Thomsen and Kettel, 1929; Kettel, 1930; Matta, 1937; Brewer, 1937; Jakobowicz and Bryce, 1941; Greval, Chandra, and Woodhead, 1941). The somewhat higher titres recorded in this series are presumably due to differences in technique.

Reports of Cases 1 to 8 (Table II)

The first three recipients were volunteers from the Depot staff. All were in good health.

Case 1.—Female aged 23, group A, weight 10½ st.; received in 20 minutes 500 c.cm. of group O serum (anti-A titre 1,024). Her inhibition index was 4. Results: no reaction; no intravascular agglutination or haemoglobinaemia; inhibition index reduced from 4 to 2; rise of bilirubin from 0.1 mg. to 0.8 mg./100 c.cm. at the end of 5 hours. *Comments:* The recipient apparently absorbed without harm 500 c.cm. of high titre serum, the equivalent of a little under a litre of whole blood. The rise of serum bilirubin in this case is of doubtful significance, as a similar rise was produced in the recipient by a later transfusion of 450 c.cm. of iso-agglutinin-free serum.

TABLE II.—*Transfusion of Group O Serum into A Recipients. A Summary of the Chief Findings in Cases 1 to 8*

Case No.	Serum given		Haemoglobinaemia	Intrav. Agglut.	Haemoglobinuria	Serum Bilirubin (mg./100 c.cm.)		Inhibn. Index		Comments
	Titre	Vol. (c.cm.)				Bef.	Aft.*	Bef.	Aft.†	
1	1,024	500	—	—	—	0·1	0·8	4	2	
2	2,048	500	—	+	—	0·5	4·0	2	2	
3	1,024	500	—	—	—	0·8	4·0	2	0	
4	8,192	150	++	—	+	0·4	3·8	0	0	Pain in back +++; fall in Hb
5	2,048	350	—	—	—	0·0	2·2	2	2	Rigor
6	8,192	30	—	—	—	0·5	2·7	4	4	Pain in back
7	8,192	130	Tr.	vw	—	0·0	1·7	2	2	Pain in back, nausea, and colic
8	16,384	450	+	vw	—	0·2	3·2	0	0	Restless; nausea

* 4 to 5 hours after end of transfusion. † Immediately after transfusion.

Case 2.—Female aged 24, group A, weight 8½ st.; received in 25 minutes 500 c.cm. of group O serum (anti-A titre 2,048). Her inhibition index was less than 2. Results: a slight pyrexia to 99.5° F. half an hour after transfusion, but no subjective symptoms; intravascular agglutinates with clumps of up to 10 cells, many deformed and discoloured, present in large numbers immediately after transfusion and persisting for two hours, but no haemoglobinaemia; serum bilirubin rose from 0.5 mg. to a maximum of 4 mg./100 c.cm. five hours later, falling to 1.1 mg. in 24 hours; no change in inhibition index.

Case 3.—Male aged 20, group A, weight 9½ st.; received in 25 minutes 500 c.cm. of group O serum (anti-A titre 1,024). Inhibition index 2. Towards the end of the transfusion he complained of a slight headache and mild "muscular" pains in the back; otherwise no abnormal symptoms or signs were recorded. Results: no intravascular agglutinates or haemoglobinaemia; serum bilirubin rose from 0.8 mg. to 4 mg./100 c.cm. 5 hours later; slight reduction in plasma agglutinogen.

Case 4.—Female aged 54, group A, weight 8 st.; recovering from a haematemesis. During the previous month she had received without incident 4 transfusions of group O blood, raising her haemoglobin to 82%. She had no detectable plasma agglutinogen. After she had been given 150 c.cm. of group O serum (anti-A titre 8,192) in 10 minutes she complained of pins-and-needles in both legs. The transfusion was stopped, but back pain developed and quickly became severe; it was mid-sacral and aching, but with sudden exacerbations. It was associated with skin pallor and coldness, but the pulse remained steady. The pain lasted about 15 minutes. She then felt normal except for a headache. A venous sample taken half an hour after the end of transfusion showed a plasma tinged with haemoglobin, but no agglutinates were found in a citrate suspension. A slight trace of haemoglobin was still present in the plasma at the end of 5 hours. Both post-transfusion samples were weakly positive to Schumm's test, but methaemalbumin could not be detected in either. Haemoglobin was present in specimens of urine passed 1, 3, and 6 hours after the transfusion; altogether about 1/4 g. was excreted. The serum bilirubin was 0.4 mg./100 c.cm. before transfusion, 0.9 mg. half an hour afterwards, and 3.8 mg. at the end of 5 hours. The patient's urine was kept alkaline and she was given abundant fluids by mouth. Her urinary output remained good, and subsequently she appeared none the worse for the reaction. Five days later the haemoglobin was 72%.

Case 5.—Female aged 23, group A, weight 9 st.; recovering from a moderate post-partum haemorrhage. Haemoglobin 10 days after delivery was 66%. Inhibition index between 2 and 4. She was given in 25 minutes 350 c.cm. of group O serum (anti-A titre 2,048). She then had a rigor lasting for 15 minutes, and the transfusion was stopped. At the end of the rigor a venous sample showed no agglutinates or haemoglobinaemia; the serum bilirubin had risen, however, from *nil* to 1.6 mg./100 c.cm., reaching 2.2 mg. at the end of 5 hours. The inhibition index after transfusion was 2. Five hours after this transfusion she was given 1,000 c.cm. of group O concentrated red cell suspension. Haemoglobin then rose to 88%.

Case 6.—Female aged 20, group A, weight 8 st.; primipara one month from term suffering from pyelitis of pregnancy. Haemoglobin 66%; inhibition index 4. She was given group O serum (anti-A titre 8,192). After she had received 30 c.cm. in 3 minutes she complained of throbbing pain in the back of the neck, passing down to the bottom of the spine, also a tight feeling in the neck and difficulty in breathing. No bronchial spasm could be detected. The blood pressure rose from 110/70 to 140/70. The pain lasted for about 10 minutes, gradually fading from above downwards. No agglutinates or haemoglobinaemia were found in a venous sample 15 minutes after the transfusion had been stopped. The serum bilirubin rose from 0.5 mg./100 c.cm. to 2.7 mg. at the end of 5 hours. The plasma agglutinogen was perhaps slightly reduced. Five hours after the serum she was given 1,000 c.cm. of group O concentrated red cell suspension. This raised her haemoglobin to 90%.

Case 7.—Female aged 43, group A, weight 8½ st.; a case of menorrhagia. Haemoglobin 54%. Only traces of agglutinogen were present in her plasma. The serum given to the previous case was used again here after refiltration (anti-A titre still 8,192). After 20 c.cm. had been given in 10 minutes the patient complained of a stitch-like pain in the small of the back, shooting down to the thighs and accompanied by jerky spasms of the back muscles, declared to be involuntary. The transfusion was stopped, and the symptoms subsided in 5 minutes. The transfusion was restarted at a very slow rate. Half an hour later, when she had received 130 c.cm., she had a spasm of abdominal pain and asked for a bed-pan. She also felt shivery and complained of a steady but not severe pain across the small of the back. Her face had become paler and she felt nauseated and uncomfortable. The transfusion was stopped. A venous sample showed a plasma tinged with haemoglobin; a number of small red cell agglutinates were visible, the largest clumps containing only 3 to 4 cells. These had disappeared at the end of 5 hours. The serum bilirubin rose from *nil* to 1.7 mg./100 c.cm. (5-hour specimen). There was no change in the plasma agglutinogen and no haemoglobinuria. Her temperature rose to 101° F. 2 hours after the transfusion. The serum transfusion was followed 5 hours later by a transfusion of 1,000 c.cm. of a group O concentrated red cell suspension. This raised her haemoglobin to 78%.

Case 8.—Female aged 38, group A, weight 9 st., with prolapse of the uterus and hypochromic anaemia; was two weeks from term (fifth pregnancy). Haemoglobin 58%. She was given in 80 minutes 450 c.cm. group O serum (anti-A agglutinin titre 16,384, anti-A haemolysin titre 16). There was some nausea and restlessness towards the end of transfusion, and the temperature subsequently rose to 101° F. The post-transfusion sample showed intravascular agglutinates with clumps of 4 to 7 cells and a plasma strongly tinged with haemoglobin. Small agglutinates were still visible 3½ hours later, but the haemoglobinaemia had ceased. The first specimen of urine passed after the transfusion was unfortunately thrown away, but was said to look normal. There was no subsequent haemoglobinuria. The serum bilirubin rose from 0.2 mg. to 3.2 mg./100 c.cm. 3½ hours later. At the end of the 3½-hour period she was given 1,000 c.cm. of group O concentrated red cell suspension, the haemoglobin then rising to 70%.

The rise in serum bilirubin (observed in every case) and the haemoglobinaemia and/or intravascular agglutination (seen in 4 cases) suggested that abnormal red cell destruction was taking place. In order to obtain more evidence the investigation was continued, red cell counts and haemoglobin and haematocrit readings being made on each subsequent case for several days after transfusion (Table III).

Reports of Cases 9 to 16

Case 9.—Female aged 46, group A, weight 6½ st.; suffering from carcinomatosis secondary to a breast cancer removed 4 years previously. Inhibition index *nil*. She was given in 50 minutes 400 c.cm. of group O serum (anti-A titre 512). During the transfusion there was transitory pain behind the knees. She subsequently felt uncomfortable and nauseated. Venous samples at the end of the transfusion showed traces of haemoglobinaemia and marked intravascular agglutination with clumps of 8 to 10 cells. The serum bilirubin rose from 0.2 mg. to 2.4 mg./100 c.cm. at the end of 3½ hours. It took two weeks to return to normal, possibly in part because of impaired hepatic function due to the secondary deposits of carcinoma. After 5 days it was still 1.6 mg. There was no haemoglobinuria. Seven days after transfusion the red cell count was 1.5 millions below and the haemoglobin 20% below the pre-transfusion level.

Case 9a.—Case 9, 3½ weeks later, was given 400 c.cm. of reconstituted agglutinin-free dried serum. There were no symptoms during transfusion, but afterwards the patient felt cold and shivery and complained of headache and aching pain in the legs. Serum bilirubin rose from 0.32 mg. to 0.53 mg./100 c.cm. 5 hours later. Four days after transfusion the haematological findings were substantially the same as before transfusion.

Case 10.—Male aged 32, group A, weight 10½ st. Left subphrenic abscess. Given in 60 minutes 250 c.cm. of group O plasma (anti-A titre 1,024). During the transfusion he complained of some cramp-like pain behind the knees, which subsided as the transfusion was slowed. The temperature subsequently rose to 101° F. There was no haemoglobinaemia or intravascular agglutination. Serum bilirubin, 0.4 mg./100 c.cm. before transfusion, was raised above this level for the next 5 days, reaching a maximum of 0.7 mg. on the third day. The red cell count fell by half a million immediately and by a further 1½ millions during the next 4 days, with a total drop of 24% in haemoglobin and 12% in the haematocrit reading.

Case 10a.—Case 10, two weeks later, was given in 45 minutes 350 c.cm. of group O plasma (anti-A titre 32). There was no reaction. The haematological findings were practically unchanged, both at the time and in the next 4 days; the serum bilirubin—0.4 mg./100 c.cm. before transfusion—fell gradually to 0.2 mg. 4 days later.

Case 11.—Male aged 57, group A, weight 9 st. Carcinoma of colon, ischio-rectal abscess, and infection of urinary tract. Inhibition index *nil*. He was given group O serum (anti-A titre 2,048). After receiving 75 c.cm. in 10 minutes he complained of constricting pains around the lower chest, and pain in the

TABLE III.—*Haematological Findings in Plasma and Serum Transfusions. Low Titre Transfusions (above Double Line) contrasted with High Titre Transfusions (below)*

Case No. (Amount) (Titre)		On Day of Transfusion			Days after Transfusion					
		Before	Immediately After	5 Hours After	1	2	3	4	5	7
9a (400 c.cm.) (0)	R.B.C.	4·15	3·85	4·10	3·65	3·60		4·25	4·35	
	Hb	86	78	82	76	76	76	84	84	
	Hmt.	38·2	33·2	37·0	36·0	34·0	36·2	38·0	37·5	
	Bilirubin	0·32	0·40	0·53	0·32	0·20		0·20	0·25	
10a (350 c.cm.) (32)	R.B.C.	3·25	3·10	3·25	3·45			3·50		
	Hb	58	58	57	60	61		62		
	Hmt.	29·0	26·5	27·5	29·2	31·5		31·0		
	Bilirubin	0·40	0·40	0·32	0·28	0·20		0·20		
11a (450 c.cm.) (128)	R.B.C.	3·90	3·75	3·75	3·70		3·60		3·70	
	Hb	58	54	57	58		60		58	
	Hmt.	30·5	27·5	30·8	31·0		30·0		30·5	
	Bilirubin	<0·10	<0·10	<0·10	<0·10					
11b (225 c.cm.) (2)	R.B.C.	3·40	3·15	3·25	3·10			2·90		
	Hb	58	55	55	49	53		50		
	Hmt.	27·0	23·5	24·2	23·0	24·2		23·5		
	Bilirubin	0·20	0·20	0·20	0·20			0·20		
13 (450 c.cm.) (32)	R.B.C.	3·50		3·10	3·40	3·25			3·76	
	Hb	50		42	44	43			47	
	Hmt.	26·2		25·2	25·2	24·5			27·0	
	Bilirubin	0·12		0·64	0·20	0·14			0·10	
14 (300 c.cm.) (32)	R.B.C.	3·60			3·55				3·60	
	Hb	58			60				64	
	Hmt.	30·5			29·0				31·2	
	Bilirubin	<0·10			<0·10				<0·10	
15 (450 c.cm.) (128)	R.B.C.	2·40	1·80	1·85	1·95	1·85			2·05	
	Hb	35	30	31	32	32			34	
	Hmt.	18·0	14·8	16·5	16·5	17·0			18·2	
	Bilirubin	<0·10	0·10	0·33	0·14	0·10			0·10	
16 (400 c.cm.) (0)	R.B.C.	3·85	3·60	3·80	3·90			3·90		
	Hb	80	76	76	76			78		
	Hmt.	37·0	33·0	34·0	34·0			36·5		
	Bilirubin	<0·10	0·10	0·20	0·13			0·10		
9 (400 c.cm.) (512)	R.B.C.	5·25	4·25	4·45	4·80	4·50	4·65	4·35	4·32	3·75
	Hb	94	80	80	88	78	82	80	76	74
	Hmt.	48·0	38·2	40·5	42·5	39·5	39·0	36·2	36·0	33·0
	Bilirubin	0·20	0·32	2·40	0·80	0·80	1·60	1·76	1·60	1·00
10 (250 c.cm.) (1,024)	R.B.C.	4·05	3·55	3·65			2·55	2·00		2·20
	Hb	64	53	57	61		45	40		42
	Hmt.	29·2	24·2	23·5	24·5		19·5	17·0		18·5
	Bilirubin	0·40	0·40	0·53	0·64		0·70	0·53		0·20
11 (225 c.cm.) (2,048)	R.B.C.	3·65	2·70	2·75	2·80	3·20	3·00		3·10	
	Hb	58	46	48	51	55	53		55	
	Hmt.	28·0	23·0	24·2	24·2	25·2	25·0		26·0	
	Bilirubin	0·10		1·33	0·20	0·20	0·15		0·10	
12 (450 c.cm.) (512)	R.B.C.	4·50	3·75	3·98	3·48	3·75		3·95		
	Hb	76	67	70	61	66		70		
	Hmt.	38·0	33·2	34·2	29·5	31·5		33·2		
	Bilirubin	0·10	0·26	0·53	0·20	0·10		0·10		

R.B.C. expressed in millions per c.mm. ; Hb in % (Haldane) ; bilirubin in mg./100 c.cm.

loins, groins, thighs, and arms. The transfusion was slowed, and the pain passed off in 10 minutes. The rate was increased again and a further 150 c.cm. was given in the next 45 minutes. The pains then recurred and a rigor set in. A sample taken at the end of transfusion showed haemoglobinaemia and large numbers of agglutinates. Red cell destruction was suggested by a bilirubin rise from 0.1 mg./100 c.cm. to 1.33 mg. and by a subsequent drop in red cell count, in haemoglobin, and in the haematocrit reading. In 3 days the fall was: 650,000 R.B.C., 5% Hb, and 3% haematocrit. Apparently considerably fewer cells were destroyed than had been agglutinated.

Case 11a.—The same case ; was given 450 c.cm. group O serum (anti-A titre 128) in 30 minutes, with no reaction. There was no rise in bilirubin and nothing in the blood count to suggest red cell destruction.

Case 11b.—The same case ; 225 c.cm. of the high titre serum first given still remained. Its anti-A iso-agglutinin was neutralized by the addition of group A specific substance as prepared by the method of Witebsky. On transfusion there was a mild pyrexial reaction but no subsequent rise in bilirubin. Over the next 4 days there was some fall in the blood count (500,000 R.B.C., 8% Hb, 3.5% haematocrit), probably associated with clinical deterioration in the patient's condition.

Case 12.—Female aged 51, group A, weight 7½ st., receiving medical treatment for gastric ulcer. She was given in 50 minutes 450 c.cm. group O plasma (anti-A titre 512). Inhibition index *nil*. One hour afterwards she felt cold and shivered. She also complained of severe backache "indistinguishable from lumbago," pain between the shoulder-blades, and headache. She vomited and asked for a bed-pan. Her temperature rose to 101.8° F. After 5 hours she felt normal except for a headache. The post-transfusion blood sample showed a considerable number of agglutinates, the largest composed of 10 to 12 cells. There was no haemoglobinaemia ; serum bilirubin rose from less than 0.1 mg./100 c.cm. to 0.53 mg. 5 hours later. The red cell count fell by 3/4 million in 48 hours, rising slightly at the fourth day.

Case 13.—Female aged 48, group A, weight 8 st., having radium treatment for menorrhagia. Inhibition index *nil*. She was given in 40 minutes 450 c.cm. of group O serum (anti-A titre 32) without reaction except for a slight pyrexia to 99.8° F. and headache. Her serum bilirubin rose from 0.12 mg. to 0.64 mg./100 c.cm. (5-hour sample). The red cell count, haemoglobin, and haematocrit reading fell slightly over the next 2 days, but within 4 days had returned to the original figure. These changes are not great enough to warrant any conclusion about red cell destruction.

Case 14.—Female aged 24, group A, weight 9 st. Caesarean section 9 days previously. Haemoglobin 58%. She was given in 50 minutes 300 c.cm. of group O serum (anti-A titre 32). She then had a rigor, and the transfusion was stopped. The plasma bilirubin remained below 0.1 mg./100 c.cm. in all post-transfusion specimens ; there was nothing in the blood count to suggest red cell destruction.

Case 15.—Male aged 54, group A, weight 10 st. Carcinoma of prostate, secondary carcinomatosis of bone. He received in 20 minutes 450 c.cm. of group O serum (anti-A titre 128). There was no reaction. The serum bilirubin rose from 0.1 mg. to 0.33 mg./100 c.cm. (5-hour sample). In the next few days there was a slight fall in red cell count and to a less extent in haemoglobin and the haematocrit reading. Again no conclusion about red cell destruction can be drawn.

Case 16.—Married woman ; group O. Convalescent after Caesarean section. This patient was used as a control and

received 400 c.cm. of group B serum. Immediately after the transfusion she shivered and later felt hot. There was a transient rise in serum bilirubin, from 0.1 mg. to 0.2 mg./100 c.cm., but no change in the red cell count.

General Discussion

Clinical Reactions

Although several of the recipients developed symptoms possibly attributable to the action of these iso-agglutinins, no reactions were observed which caused more than a transient deterioration in the general condition. Even in the patient (Case 4) who developed severe lumbar pain, haemoglobinaemia, and haemoglobinuria the symptoms subsided in a few minutes except for a mild headache. The haemoglobinuria persisted for 6 hours, but there was no evidence of any impairment of renal function.

Of 12 cases receiving serum or plasma containing high titre iso-agglutinins, 5 developed a syndrome, the most striking symptoms of which were moderate or severe aching pain across the small of the back, often radiating to the thighs, constricting sensations in the neck and chest, intestinal colic, and nausea.

It is tempting to associate the symptoms produced by the sera in Cases 4 and 7 with the liberation of haemoglobin from red cells haemolysed by the anti-A iso-haemolysin. Gilligan *et al.* (1941) reported abdominal cramp with vomiting and visible peristalsis in 2 subjects after the intravenous injection of 10 g. and 16 g. of haemoglobin. O'Shaughnessy *et al.* (1939) observed chills and fever, pain in the back and loins, and constricting sensations in the chest in 2 out of 4 patients with anaemia following the intravenous injection of 10 to 50 g. of haemoglobin. Fairley (1940) recorded nausea and fever in one subject after an intravenous injection of 21 g. of haemoglobin. On the other hand, similar symptoms occurred in Case 6, in which there was no demonstrable haemoglobinaemia. Moreover, we have unpublished records of other transfusions in which agglutinin-free serum caused similar severe reactions, with intense back pain and cramp. No conclusions can therefore be drawn about the cause of these reactions. They may be due to unidentified substances in the serum.

It should be stressed that all the patients transfused in this series were fully conscious and able to report symptoms, which in some cases made it appear wise, in view of the high titre iso-agglutinin content, to stop the transfusion. This safeguard would be lacking in an unconscious patient. Moreover, all the recipients were fully alkalinized and were under constant and close observation.

Rigors (3 cases), headaches, and pyrexial reactions above 100° F. (9 cases) were also observed. These are apt to occur with transfusions not involving incompatible iso-agglutinins.

Effect on Recipient's Erythrocytes

The haematological findings in the low titre transfusions were compared with those of the high titre transfusions (Table III). It will be seen that in the low titre series, with the exception of Case 11b, the findings 4 days after were the same, within the limits of experimental error, as those before transfusion. In the high titre series, however, in Cases 9 and 10 the results are certainly, and in Cases 11 and 12 probably, significant of red cell destruction.

Damaged red cells can be removed from the circulation by the phagocytosis in the reticulo-endothelial system, with the ultimate production of bilirubin. When actual intravascular haemolysis occurs three mechanisms for the removal of the extracorpuscular haemoglobin exist: (1) absorption by the reticulo-endothelial system; (2) intravascular katabolism to methaemalbumin and its elimination by the liver; (3) renal excretion. Fairley (1940) considers that, with slight degrees of intravascular haemolysis or where only small amounts (5 g.) of haemoglobin are injected into the circulation, the first method alone is involved. Of these cases, while some showed evidence (haemoglobinaemia) of intravascular haemolysis, only one gave a positive Schumm reaction. Thus only small amounts of blood would appear to have been destroyed intravascularly, the rest by intracellular phagocytosis.

One case (No. 4) showed marked haemoglobinaemia; 2 (Nos. 2 and 12) showed intravascular agglutination alone; while 4 others (Nos. 7, 8, 9, and 11) developed both. These findings were in all cases transient, and had always disappeared after 5 hours. We believe this is the first time that intravascular agglutination and haemoglobinaemia after the transfusion of serum or plasma have been reported. It is not clear why some cases should show haemoglobinaemia without agglutination and others agglutination without haemoglobinaemia. In all 7 of these cases a substantial rise in the serum bilirubin was recorded, suggesting that abnormal red cell destruction had occurred (see Tables II and III). In Cases 9, 11, and 12 this was supported by a fall in the red cell count; in Case 4 there was a fall in haemoglobin; in the other 3 cases serial red counts and haemoglobin estimations were not done. The remaining 5 cases receiving high titre group O serum or plasma (titre 512 or over) did not show either haemoglobinaemia or intravascular agglutination, but large serum bilirubin rises were again found, suggesting that red cell destruction had occurred here also. Serial blood counts in these cases were done only in Case 10. Here there was a profound drop of 2 million red cells and a slight rise of bilirubin lasting for 4 days.

The question of the titre at which red cell damage in the recipient becomes substantial is of the greatest importance. Of two cases given serum or plasma with a titre of 512 one (Case 9) certainly and the other (Case 12) probably sustained red cell destruction. It seems, therefore, that even comparatively moderate titres—i.e., 512 by our technique—are high enough to reduce the efficacy of a transfusion designed to raise the recipient's red cell count. Roughly 40% of group O donors (Table I) have an anti-A titre of 512 or higher, and about 9% an anti-B titre of the same order. As the erythrocytes of different individuals seem to vary in their sensitivity to iso-agglutinins, and as small degrees of red cell destruction fall within the limits of experimental error, much work will have to be done before a harmless titre level can be defined. It must again be stressed that numerical titres may vary greatly with workers and technique.

Effect of Recipient's Plasma Agglutinogen

It is clear that the presence of appreciable amounts of agglutinogen in the plasma of the recipient will not completely protect his red cells from agglutination or haemolysis. Of the 6 cases with inhibition indices of not less than 2, two showed a definite reduction in plasma agglutinogen following the transfusion. The protocols of two others suggest that it was slightly reduced. In the remaining two cases no reduction was detected, although both showed intravascular agglutination. Of 4 cases, none of which had any plasma agglutinogen before transfusion measurable by the inhibition technique, all showed haemoglobinaemia or intravascular agglutination, three of them both. From these results no clear picture emerges of the part played by the plasma agglutinogen in protecting the red cells except that this protection is not absolute.

In general, therefore, when a transfusion is designed to raise the recipient's red cell count, homologous group blood should be given if possible. On the other hand, in an emergency, when restoration of the blood volume of the recipient is the chief aim, the use of the universal donor is justified. Plasma and serum are also given for this purpose. When pooled, in suitable proportions, as is the practice in the emergency blood transfusion services, the anti-A and anti-B titres rarely exceed 16, so that the iso-agglutinin content is small enough for these pools to be given with confidence to recipients of any group.

Summary

The anti-A and anti-B iso-agglutinin titres of 250 group O donors have been determined.

The transfusion of conscious recipients of group A (12 cases) with considerable volumes of group O serum or plasma containing extremely potent anti-A iso-agglutinins did not produce any reaction which could be classed as dangerous. No evidence, therefore, was obtained that "incompatible" iso-agglutinins are responsible for severe or fatal haemolytic reactions.

In all these transfusions, however, there was some evidence of red cell destruction in the recipient, with the production of symptoms in some cases.

Group O blood with an agglutinin titre of 512 and over is not recommended for recipients of other groups in non-emergency transfusions designed to raise the red cell count.

The agglutinogen present in the plasma of group A recipients is not an absolute protection against anti-A agglutinins.

The iso-agglutinin content of serum and plasma pools prepared by the techniques used at the London Blood Supply Depots can safely be ignored.

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TREATMENT OF GONORRHOEA, BALANITIS, AND ACQUIRED PHIMOSIS

BY

T. R. LLOYD JONES, Surgeon Commander R.N.
Naval Medical Registrar for Venereal Diseases, Royal Navy

1. GONORRHOEA

For the purposes of this part of the article are here described a total of 500 cases which were treated by us as out-patients from the onset of the disease. In view of the varied scales of dosage of sulphapyridine used throughout the Navy, our object was to find out what daily amount would lead to cure in a reasonable period and at the same time would not render a patient unfit to carry out his routine work. This point was regarded as most important, because it is usual in the Navy for uncomplicated cases of gonorrhoea to be kept on full duty.

All these patients have carried out their daily routine work, and, so far as our statistics show, their employment during treatment has not been to the detriment of their probable cure. As regards diet, all have been on ordinary Service food, with no restrictions whatsoever: the taboo of specified articles of diet in a community in which set meals are prepared and are eaten by all is an impossibility in practice, so our patients have not had to worry as to what they should or should not eat. This, psychologically, is probably of benefit; at all events, it has not retarded the progress of cure and has caused no abnormal gastric upsets.

TABLE I

Group	Dosage	Total Cases treated	Straightforward Cases	Cases requiring Adjuvant Treatment	Average No. of Days under Treatment		
			No.	No.	Straightforward Cases	Adjuvant	Total Cases
1	27 g. (54 tablets: 10, 8, 8, 8, 8, 6, 6)	141	111 (78·7%)	30 (21·3%)	8·6	22·4	11·5
2	29 g. (58 tablets: 12, 10, 10, 10, 8, 8)	50	41 (82·0%)	9 (18·0%)	6·8	24·8	10·0
3	27·5 g. (55 tablets: 10, 9, 9, 9, 9, 9)	154	125 (81·2%)	29 (18·8%)	7·1	13·9	8·4
4	27 g. (54 tablets: 9, 9, 9, 9, 9, 9)	155	139 (89·7%)	16 (10·3%)	6·6	16·5	7·6
Totals	—	500	416 (83·2%)	84 (16·8%)	7·3	18·6	9·2

No drugs other than sulphapyridine were given. Alcohol was prohibited. Shore leave automatically stopped, thereby largely preventing civilian contagion. No irrigations were given in the so-called straightforward cases set out in Table I. Cure was hoped for when discharge had ceased and the morning urine was clear. A provocative irrigation of silver nitrate 1 in 15,000 was then given, and the following morning a urethral and prostatic smear was taken; a few epithelial cells or pus cells were not considered a bar to discharge to duty from the "attending list."

Treatment

Group 1.—In this group (Table I) 141 cases were treated with 27 g. (54 tablets) of sulphapyridine over a period of 7 days, taken as follows: 10 tablets the first day, 8 tablets on the second, third, fourth, and fifth days, and 6 tablets on the sixth and seventh days. Of these 141 cases 30 required adjuvant treatment (Table II), 27 being mild anterior or anterior and posterior infections, responding to irrigations and massage, etc. The case of arthritis was of doubtful gonococcal origin and lasted only 3 days, while both the epididymitis and the Tyson's gland infection were established before treatment by us. These 3 cases were placed on the light-duty list for a short time only. Whilst under treatment no cases of epididymitis occurred in any of the groups. Ten toxic reactions were recorded (Table III), a rate of 7.1%.

TABLE II

Group	Anterior and Posterior Infections	Arthritis	Epididymitis	Periurethral Abscess	Tyson's Gland Infection	Relapses
1	27	1	1	—	1	4
2	8	—	1	—	—	—
3	26	—	2	1	—	3
4	13	—	1	2	—	5
Totals	74	1	5	3	1	12

TABLE III

Group	Mild Gastric Upsets	Headaches	Cyanosis	Rashes
1	2	—	1	7
2	6	—	—	3
3	13	2	—	1
4	1	—	—	—
Totals	22	2	1	11

Group 2.—It was thought that if the daily dosage could be slightly increased and the total days reduced one could perhaps succeed in getting cases off the attending list more quickly. Therefore a dosage of 29 g. (58 tablets) given over a period of 6 days was tried—12 tablets on the first day, 10 on each of the second, third, and fourth days, and 8 on the fifth and sixth days. But it was found that, although the average number of days under treatment was slightly reduced, there was a definite increase in gastric upsets and rashes, a total of 9 cases occurring out of 50 in this group, averaging 18%, as compared with 10 cases out of 141, averaging 7.1%, in Group 1.

Group 3.—Because of this increase in toxic symptoms in Group 2 it was decided to reduce the dosage but to adhere to the same number of days' treatment; consequently a total dosage of 27.5 g. (55 tablets), 1.5 g. less than Group 2, was tried. On the first day 10 tablets were given, and 9 on each of the remaining days. The result of this survey gave 13 gastric upsets, 2 mild headaches, and 1 rash, a total of 16 cases in 154, averaging 10.4%—slightly higher than Group 1, but much less than Group 2. It was noticed in this group that, although the total dosage was slightly decreased, the average number of days' treatment before presumed cure had also definitely decreased.

Group 4.—As the results in Group 3 had been exceedingly good it was thought that it might be of benefit to reduce the first day's dosage slightly to see if gastric upsets were lessened, as it