BIBLIOGRAPHY

- Ardashnikov, S. N. (1934). *Proc. Maxim Gorky Med. Biol. Res. Inst.*, 3, 168 (in Russian).
 — (1937). *J. Hyg., Camb.*, 37, 286 (contains further references).
 Bauer, J. (1924). *Die konstitutionelle Disposition zu inneren Krankheiten*, Berlin.
 Levin, A. E., and Kuchur, B. A. (1937). *Lancet*, 1, 204.
 Martynova, R. P. "Diagnostic Reactions in Relatives of Cancer Patients" (in press).
 Pesikova, L. N., "On the Aetiology and Pathogeny of Diabetes Insipidus." Dissertation.
 Ryvkin, I. A., Malkova, N. N., and Kantonova, A. I. (1936). *Neurology and Genetics*, 1, 305 (Russian, English summary).
 Schmegler, F. E., and Krause, F. (1937). *Klin. Wschr.*, 16, 156.
 Thompson, W. P. (1931). *Amer. J. med. Sci.*, 182, 334.

PRODUCTION OF Rh AGGLUTININS ANTI-C AND ANTI-E BY ARTIFICIAL IMMUNIZATION OF VOLUNTEER DONORS

BY

J. J. VAN LOGHEM, M.D.

With the Technical Assistance of Miss M. v. d. HART

(From the Centraal Laboratorium van den
Bloedtransfusiendienst, Binnengasthuis, Amsterdam)

The Rh agglutinins anti-C and anti-E used for Rh subgroup typing have until now been obtained from mothers and patients with agglutinins induced by Rh antagonism. It is possible to increase the titre of these antibodies by artificial immunization with the specific antigens C or E (R' or R'' cells). If these sera are obtained from Rh-negative persons they usually contain incomplete anti-D antibodies as well.

In order to obtain pure anti-C and anti-E agglutinins without incomplete anti-D the following experiments were performed in the Central Laboratory of the Blood Transfusion Service in Amsterdam.

Rh-negative male volunteer donors were injected with R'r (Cde/cde) and with R''r (cdE/cde) cells. This procedure is very simple.

F. K., a male volunteer donor aged 18, blood group O rr, was treated in the following way.

He received five injections of 2 ml. of R''r cells of group O, followed by seven injections of 0.25 ml. (injections twice a

TABLE I.—Donor F. K. (Blood Group O rr)

Date	No. of Inj.	Quantity of R' Cells given I.V. (ml.)	Rh Antibodies			
			Early-immune		Hyperimmune	
			E	D	E	D
21/11/46	1	2.0				
25/11/46	2	2.0				
28/11/46	3	2.0				
2/12/46	4	2.0				
5/12/46	5	2.0				
9/12/46	6	0.25				
12/12/46	7	0.25				
17/12/46	8	0.25				
20/12/46	9	0.25				
24/12/46	10	0.25				
30/12/46	11	0.25				
6/1/47	12	0.25				
13/2/47	13	0.25				
17/2/47	14	0.25				
20/2/47	15	0.25				
3/3/47	16	0.25				
7/3/47	17	0.25				
8/3/47			1:16*	—	1:16	—
10/3/47	18	0.10	1:64†	—	?	—
17/4/47	19	0.10				
24/4/47	20	0.05				
28/4/47	21	1.0	1:64†	—	?	—
3/6/47	22	1.0				
23/6/47	23	0.5				
21/7/47	24	0.5				
8/9/47	25	1.0				
18/9/47	26	0.5	1:32		1:64	1:128†

* Visible macroscopically. † Weakly positive macroscopically.

week). After a period of rest for nearly six weeks five further injections of 0.25 ml. of blood were given with approximately the same time intervals. During this period no Rh agglutinins were present in his serum. After the last injection of 0.25 ml. of R''r blood (17th injection from the beginning) a chill with rise of temperature resulted. Rh agglutinins type anti-E with titre 1:16 could now be demonstrated (the titre in saline was the same as in albumin). After a new series of nine injections during 27 weeks the titre increased to 1:32 in saline and to 1:128 in albumin (see Table I).

In this way it is proved that it is possible to produce a pure anti-E antibody by repeated injections with R'' cells. Besides the complete anti-E (early-immune) antibodies, it seems that incomplete (hyperimmune) antibodies type anti-E were also formed.

Encouraged by this success five other male volunteer donors were injected with R' cells, group O (donor Sch. R'R' and donor Dam. R'r). Practically the same procedure of immunization as in the first case was followed. Only one of these donors seemed to be sensitive enough to produce Rh antibodies.

G. de J., a male volunteer donor aged 33, blood group A rr (see Table II). After the 13th injection Rh agglutinins were

TABLE II.—Donor G. de J. (Blood Group O rr)

Date	No. of Inj.	Quantity of R' Cells given I.V. (ml.)	Rh Antibodies				Cell Suspensions R'
			Early-immune		Hyperimmune		
			C	D	C	D	
11/3/47	1	2.0					Sch.
14/3/47	2	1.0					"
17/3/47	3	1.0					"
20/3/47	4	0.25					"
24/3/47	5	0.25					"
27/3/47	6	0.25					"
3/4/47	7	0.25					"
8/4/47	8	0.25					"
10/4/47	9	0.25					"
19/5/47	10	0.25					"
27/5/47	11	0.25					"
2/6/47	12	0.25					"
5/6/47	13	0.25					Dam Sch.
9/6/47	14	0.25	1:2				Dam.
12/6/47	15	0.25					Sch.
16/6/47	16	0.25					Dam.
19/6/47	17	0.25					Sch.
23/6/47	18	0.25	1:8*		—	1:8	Dam.
3/7/47	19	0.25					Sch.
10/7/47	20	0.25					"
21/7/47	21	0.25					Dam.
24/7/47	22	0.25					Sch.
28/7/47	23	0.25					Dam.
3/8/47	24	0.25					Sch.
14/8/47	25	0.25					Dam.
21/8/47	26	0.25					"
25/8/47	27	0.25					"
29/8/47	28	0.25					"
4/9/47	29	0.25	1:4		1:16		"
11/9/47	30	0.25					"
19/9/47	31	0.25					"
22/9/47	32	0.25	1:4		1:32	1:8	"

* Visible macroscopically.

present in his serum (titre 1:2). After the 17th injection with R' cells of group O the titre increased to 1:8 (visible macroscopically), 1:16 weakly positive, type anti-C, with incomplete antibodies type anti-D, titre 1:8 (albumin test) without incomplete (or hyperimmune) anti-C. After a new series of 11 injections the titre of anti-C in saline fell to 1:4, but increased in albumin to 1:16. After four more injections the titre in saline was unchanged, but in albumin it increased to 1:32.

Thus it appears to be necessary to stop the immunization at the right moment in order to prevent the production of the hyperimmune antibodies, which sometimes cause a decrease in titre of the early-immune antibodies.

In cases of erythroblastosis foetalis Diamond demonstrated that after repeated immunizations Rh agglutinins (early-immune antibodies) are replaced by (or changed into) Rh-hyperimmune antibodies. In these two cases of artificial immunization these findings could be confirmed.

Four other donors treated in the same way have failed to produce Rh antibodies.

So long as there are found anti-D antibodies of the incomplete type only artificial immunization of male volunteer donors is a useful method in preparing anti-C and anti-E agglutinins.

Note on the blood of the two donors to G. de J. ; by R. R. Race, M.R.C.S., L.R.C.P. (Medical Research Council, Blood Group Research Unit, Lister Institute, London)

The finding of some anti-D as well as the hoped-for anti-C in the serum of volunteer G. de J. was of considerable theoretical interest, for it appeared that the antigen C might be capable of stimulating the antibody anti-D. It therefore became important to test the cells of the two R' donors as fully as possible in order to detect the antigen D^u if it should be present. Agglutination tests gave the following results :

	Anti-						
	C	C ^w	c	D	E	e	
Donor Dam. . .	+	-	+	-	-	+	i.e., apparently Cde/cde (R'r) Cde/Cde (R'R')
Donor Sch. . .	+	-	-	-	-	+	

When these two bloods were further tested with many anti-D sera (using the saline, albumin, and antiglobulin techniques) some of the reactions were positive ; that is to say, the samples should more properly be called CD^ue/cde and CD^ue/Cde. There are several grades of the D^u antigen distinguishable by the different proportion of anti-D sera with which they react. The D^u antigen in these two bloods was low on the scale.

In view of the close relationship between the antigen D and the antigen D^u, it seems reasonable to attribute the anti-D antibody in the serum of the volunteer to the D^u antigen present in the blood of both his donors.

Medical Memoranda

Two Cases of Fugue after Mepacrine Administration

Although it is by no means certain that the administration of mepacrine ("atebrin") was the cause of the fugues here described it is felt that these case notes should be placed on record because of the striking sequel in both cases to moderately heavy doses of the drug and because of the tragic circumstances which ensued in Case 1. Both cases occurred in the Anglo-Egyptian Sudan.

CASE REPORTS

Case 1.—The patient, a happily married British woman aged 32, with two children, had no family or personal history of mental disorder and no private worries. She was first seen on Oct. 16, 1945, by a Sudanese doctor in an out-station. She was suffering from fever (temperature 103° F.=39.4° C.) and severe headache. M.T. malaria was diagnosed by blood examination, and the following course of mepacrine was given: First day, 0.3 g. followed by 0.3 g. three hours later; second to fourth days (inclusive), 0.2 g. night and morning—total, 1.8 g. The condition responded quickly to treatment, and on Oct. 19 the patient felt fit and well. She asked permission to play tennis, but this was refused. That night, after dinner, she and her husband retired to bed at about 9.30 o'clock. They chatted for a while in bed and the patient appeared to be her normal cheerful self. The following morning the husband awoke to find that his wife had disappeared. Shortly afterwards some natives arrived with the news that her body had been found in the river. Post-mortem examination showed that death had been due to drowning, and there were no other abnormal findings.

Case 2.—A British man aged 47, with no family history of mental disorder, had suffered from claustrophobia after the 1914-18 war due to having been buried alive three times by shell bursts, but the condition had completely cleared up by 1924. I saw him on Nov. 3, 1945, when he was suffering from fever (temperature 101° F.=38.3° C.) and pains in the back and bones. M.T. malaria was diagnosed by blood examination and he was put on the following course of treatment: First day, 0.2 g. of mepacrine followed by 0.2 g. after four hours; second to fifth days (inclusive), 0.1 g. of mepacrine t.d.s.; sixth to tenth days (inclusive), 10 gr. (0.65 g.) of quinine bihydrochloride t.d.s. On the evening of the sixth day of treatment the patient felt sick after taking quinine. On the seventh day, without medical advice, he decided to revert to mepacrine treatment and proceeded to take 0.1 g. t.d.s. On the evening of the ninth day—i.e., after he had taken a total of 2.4 g. of mepacrine, he went to his bath at his usual hour of 6 p.m. While sitting in the bath he felt a "feeling of unreality coming over him." The next thing he knew was that he was sitting in his dining-room naked and wet. The interval of amnesia had lasted for about half an hour. Mepacrine was at once stopped and no further ill effects occurred.

I have to acknowledge with thanks the permission of the Director, Sudan Medical Service, to publish these notes.

D. R. MACDONALD, M.B., Ch.B., M.R.C.P.,
Sudan Medical Service.

Unusual Case of Calcification of the Myocardium

Calcification of the heart to a great extent, without a previous history of pericarditis or other heart disease, is rare, and the facts revealed by the following case report are, I believe, unusual.

CASE HISTORY

The patient was admitted to hospital when 35 years of age, suffering from dementia and mental deficiency. It was stated by his relatives that he had not had any previous illnesses. His clinical record during the whole of his fifteen years in hospital showed that at all times he suffered from slight dyspnoea and cyanosis of both hands and feet, and occasionally he had swelling of the lower extremities. Attendants who were in close contact with the patient during the whole of his hospital life state that although he never actually worked he was, quite capable of walking about and attending to his own needs. During the last four years he was confined to his bed, but got up every day for toilet purposes. One morning, fifteen years after admission, just after he had returned to bed he suddenly collapsed and died. He was then 49 years of age.

At necropsy the main pathological changes were found in his heart. This organ was enlarged (weight 360 g.) and felt extremely hard, in fact the usual post-mortem scissors would not cut into its substance, and bone forceps had to be used. The pericardium was fused completely over the whole surface of the left ventricle. The latter on being opened was found to contain ante-mortem clot (see illustration). In the lower two-thirds of the left ventricle there was no muscle tissue left at all; it was simply an ossified wall, which was obviously the result of a fusing of the pericardium and endocardium. This wall had a maximal thickness of 7 mm. The only area where muscular tissue was left was in the upper part of the left ventricle and a very small amount of its apex. The muscular wall of the right ventricle was somewhat hypertrophied and showed no signs of calcification. There was no gross pathological alteration in the valves, nor did the aorta or other arteries exhibit any atheroma or other changes.

SELWYN HEWITT, M.B., B.S., B.Hy., D.P.H.