FURTHER EXPERIMENTAL STUDIES ON THE PREVENTION OF Rh HAEMOLYTIC DISEASE

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In a previous report (Finn *et al.*, 1961) we gave reasons for thinking that the rapid removal of Rh-positive foetal erythrocytes from the circulation of a mother who was Rh-negative would prevent her from becoming immunized and producing Rh antibodies. We have now investigated the matter further, and the present paper describes the completed results of the earlier work (Experiment I) and then gives details of some subsequent observations (Experiments II and III). The reasoning involved and the scope of the investigations are first discussed.

Levine (1943) demonstrated the marked degree of protection against Rh haemolytic disease afforded by ABO incompatibility between mother and foetus. We agreed with Race and Sanger (1950) that the probable mechanism lay in the rapid destruction of the incompatible foetal cells in the circulation by the anti-A and anti-B. Experiment I was therefore designed to determine whether immunization resulting from injection of ABO compatible Rh-positive blood into Rh-negative male volunteers could be prevented by the infusion of plasma containing high-titre saline-reacting (*complete*) anti-D antibodies, simulating the effect of anti-A and anti-B. Some initial results of this experiment have been briefly reported (Finn *et al.*, 1962). It was found that, compared with control subjects, antibody formation was enhanced rather than prevented and we noted that in some cases nearly half the injected cells survived in the circulation for over a week, though at that time free complete anti-D could be detected in the recipients' sera.

On the basis of these results a second approach which forms the substance of Experiment II was tried. Stern *et al.* (1961) had shown that prior *in vitro* coating of Rh-positive erythrocytes with *incomplete* anti-D would prevent antibody formation after subsequent injection into Rh-negative males. It was thought that the mechanism operating here might be the complete blocking of the D-antigen sites by incomplete antibody. Mollison (1959) had shown that this was associated with the rapid clearing of such cells from the circulation. Our experiment thus involved the use of plasma containing high titres of incomplete anti-D antibodies and a study of its effectiveness in preventing Rh immunization by previously injected Rh-positive blood.

Further studies were carried out in Baltimore (Experiment III) to elucidate the relationships between differing amounts and types of Rh antibody, the rapidity of clearance of injected Rh-positive cells, and subsequent immunization.

Materials and Methods

The experiments were carried out on groups of Rh-negative male volunteers. Those in Liverpool were blood donors and those in Baltimore were inmates of the Maryland State Penitentiary. The method of giving the blood and plasma was the same as that described in Finn *et al.* (1961).

In Experiments I and II the men were dealt with in groups of six; as a rule, three received 5 ml. of Rh-positive blood and then half an hour later were given the antibody-containing plasma, while the other three received only the Rh-positive blood and thus acted as controls. The details of what was given to each of the 24 men in Experiment I are shown in Table I and to each of the 42 men in Experiment II in Table II. It was the aim in the second experiment to use 50 ml. of plasma with a high titre of incomplete antibody, thus attempting to achieve rapid clearance of injected blood, but because of practical difficulties this dose was not always possible.

TABLE I.—*Details of Volume and Type of Anti-D Sera Used in Experiment I*

Group	Volunteer No.	1st Stimulus			2nd Stimulus		
		Volume (ml.)	Titre		Volume (ml.)	Titre	
			Saline	Albumin		Saline	Albumin
L.I	1, 2, and 3	10	128	128	10	1,024	1,024
	4, 5, and 6	Nil	—	—	Nil	—	—
L.II	1, 2, and 3	20	512	512	20	64	64
	4, 5, and 6	Nil	—	—	Nil	—	—
L.III	1, 2, 3, and 4	20	512	512	20	64	64
	5 and 6	Nil	—	—	Nil	—	—
L.IV	1, 2, and 3	20	0	16	15	64	64
	4, 5, and 6	Nil	—	—	Nil	—	—

Notes.—The volume of Rh-positive blood given at each stimulus was 5 ml. In L.I the Rh type was CDe cde and in L.II, III, and IV it was cDE/cde. There was an interval of about three months between the two stimuli. L.III 4 did not receive a second stimulus. Groups L.III and IV received two doses of 10 ml. of antiserum with a week between the doses except for L.III 3, whose first dose was 5 ml. of antiserum.

In the further clearance studies of Experiment III the men were again dealt with in groups of six, but instead of matching "treated" subjects with controls three were given 10 ml. and the other three 50 ml. of the same plasma 20 minutes after the blood. Most of the subjects were given 5 ml. of Rh-positive blood, but 10 ml. was used in a few instances to see if this amount could be cleared as rapidly as 5 ml. The details of what each volunteer received are shown in Table III. The main purpose of these studies was to obtain information on how to clear Rh-positive cells from the circulation quickly, but the subjects were also tested for the development of immune antibodies to obtain more detailed information on the relationship between clearance and subsequent immunization.

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TABLE II.—*Details of Rh Type of Blood Given to all Six Men in each Group, and Titre of Anti-D Sera Given to Three "Treated" Men in each Group at each Stimulus in Experiment II*

Group and Volunteer Nos.	1st Stimulus			2nd Stimulus			3rd Stimulus			4th Stimulus		
	Rh Type	Anti-D Titres		Rh Type	Anti-D Titres		Rh Type	Anti-D Titres		Rh Type	Anti-D Titres	
		Saline	Albumin		Saline	Albumin		Saline	Albumin		Saline	Albumin
L.V 1–6 ..	CDe/cde	0	8	CDe/cde	0	8	CDe/cde	0	512	CDe/cde	0	256
L.VI 1–6 ..	CDe/cde	1	16	CDe/cde	1	2,048	cDE/cde	0	1,024	—	—	—
L.VII 1–6 ..	CDe/cde	1	128	CDe/cde	2	2,048	cDE/cde	0	256	—	—	—
L.VIII 1–6 ..	CDe/cDE	2	1,024	cDE/cde	2	1,024	CDe/cde	1	512	cDE/cde	0	1,024
L.IX 1–6 ..	CDe/cDE	1	512	cDE/cde	4	512	CDe/cde	2	1,024	cDE/cde	0	512
B.I 1–6 ..	CDe/cDE	2	1,024*	CDe/cDE	0	128*	CDe/cDE	1	1,024*	CDe/CDe	0	512*
B.II 1–6 ..	cDE/cde	2	512*	CDe/cDE	0	256*	CDe/cDE	0	2,048*	CDe/CDe	0	256*

* In groups B.I and B.II the incomplete titre was measured by the quantitative indirect Coombs test and not in albumin.

Notes.—The volume of Rh-positive blood given at each stimulus was 5 ml. The volume of anti-D given to the three "treated" men (Nos. 1, 2, and 3) in each group was 50 ml. at each stimulus until immune antibody developed, with the following exceptions: group L.VIII received 35 ml. at first stimulus; L.VII 1 received only 30 ml. at his second stimulus; L.VIII 3 received 36 ml. at his first stimulus; and B.II 2 received 44 ml. at his first stimulus. The time interval between stimuli varied from two to five months, but was usually three months, with the exception of groups B.I and B.II, where it was usually about one month.

TABLE III.—*Volume and Titre of Anti-D Given to Men in Experiment III*

Group	Volunteer Nos.	Volume of Anti-D (ml.)	1st Stimulus Anti-D Titre		2nd Stimulus Anti-D Titre		3rd Stimulus Anti-D Titre		4th Stimulus Anti-D Titre	
			Saline	I.C.T.	Saline	I.C.T.	Saline	I.C.T.	Saline	I.C.T.
B.III	1, 2, and 3	50	8	1,024	1	512	0	1,024	0	256
	4, 5, and 6	10								
B.IV	1, 2, and 3	50	0	8	0	64	64	1,024	4	1,024
	4, 5, and 6	10								
B.V	1, 2, and 3	50	1	8	1	4	128	2,048	128	2,048
	4, 5, and 6	10								
B.VI	1 and 2	50	2	512	0	256	0	2,048		
	3 and 4	10	—	—	—	—	—	—		
	5 and 6	Nil								
B.VII	1, 2, and 3	50	32	512	1	1,024				
	4, 5, and 6	Nil	—	—	—	—				

Notes—The Rh type of blood given was CDe/cDE in all groups and at each stimulus with the exception of B.III at the third stimulus, B.IV at the second and third stimuli, and B.VI at the first stimulus, when it was cDE/cde. The volume of Rh-positive blood injected was 5 ml. in groups B.III, B.IV, and B.V and 10 ml. in groups B.VI and B.VII. The time interval between stimuli varied from four to eight weeks, but was usually six weeks. Volunteers B.V 4 and 5 and B.VI 4 did not receive any anti-D serum at their second, third, and fourth stimuli. Volunteer B.VI 3 received 10 ml. anti-D at first stimulus and 50 ml. at second and third stimuli. Volunteers B.VI 1 and 5 received only the first stimulus. Volunteer B.V 2 received only two stimuli.

The anti-D-containing plasma was obtained either from immunized women or from our male volunteers who had become immunized during the course of the experiments. In all the Baltimore and many of the Liverpool studies, the Rh-positive cells were tagged with ^{51}Cr so that it was possible to tell how rapidly the cells had been removed from the circulation. At various times after an initial four-weeks period blood was taken and examined for the presence of immune anti-D. If it was not detected a further injection of Rh-positive blood was given followed by antibody in the "treated" groups of volunteers.

In some cases where the larger volumes of plasma containing high titres of incomplete antibody were used, the persistence of passive immunity was observed for many weeks. By following the titre of this antibody and by testing for the presence of saline antibodies it was always possible eventually to distinguish between passive and active immunization.

Results

Experiment I

Table IV shows the results in the four groups of men who received 5 ml. of Rh-positive blood followed by 10–20 ml. of plasma and in their controls. It can be seen that 8 out of the 13 volunteers receiving anti-D and 1 out of the 11 controls produced immune anti-D after two stimulations. One of the "treated" volunteers (L.III 4) withdrew after the first stimulus and before he could be followed up for antibody formation. Excluding this case, the data can be arranged as a 2×2 table, giving the number of volunteers who did and did not become immunized in the treated and control groups. Using Fisher's exact test for such tables and taking into account both tails of the distribution, we found that the probability of getting by chance alone a difference

TABLE IV.—*Immune Antibody Formation (A) in Experiment I, and Results of ^{51}Cr Studies Where These Were Carried Out (See Also Table I)*

Group and Volunteer Numbers		Percentage of Rh-positive Cells Surviving 48 Hours after Injection	
		1st Stimulus	2nd Stimulus
L.I	1	13 A	56 A
	2	48	
	3	40 A	
	4	98	100
	5	94	89
	6	106	77
L.II	1	43	—
	2	25	—
	3	34	—
	4	93	—
	5	91	—
	6	69	—
L.III	1	53 A	66 A
	2	66	
	3	36 A	
	4	37	*
	5	93	—
	6	98 A	
L.IV	1	91	— A
	2	91	—
	3	82 A	
	4	79	—
	5	82	—
	6	90	—

* Volunteer L.III 4 did not receive a second stimulus and was not tested for immune antibody formation.

Note.—In groups L.I, L.II, and L.IV Nos. 1, 2, and 3 were given anti-D and Nos. 4, 5, and 6 were controls. In group L.III Nos. 1–4 were "treated" and Nos. 5 and 6 were controls.

between the two groups as great as or greater than that observed is 0.01.

Thus this experiment provides strong evidence that administering 10–20 ml. of these particular plasmas enhances the likelihood of inducing immune antibody formation.

Experiment II

Table V shows the results in the men given 35–50 ml. of plasma containing predominantly incomplete anti-D and in their controls. Three of the 21 treated and 11 of the 21 controls were immunized. Here the exact test shows that the probability of getting by chance alone a difference between the two groups as great as or greater than that observed is 0.02.

This analysis thus indicates that individuals treated with 35–50 ml. of the types of plasma used here are significantly less likely to produce immune antibodies than their controls, in sharp contrast to the results of Experiment I.

TABLE V.—*Immune Antibody Formation (A) in Experiment II (See Also Table II) and the Results of ^{51}Cr Studies Where These Were Carried Out. (In the Liverpool Series it Was the Usual Practice from Group L.V. Onwards Only to Tag One "Treated" and One "Untreated" in Each Group)*

Group and Volunteer Nos.	Percentage of Rh-positive Cells Surviving 24 Hours after Injection			
	1st Stimulus	2nd Stimulus	3rd Stimulus	4th Stimulus
L.V 1	3	2	—	4
L.V 2	1	0	—	0
L.V 3	4	1	—	19
L.V 4	98	—	—	—
L.V 5	84	—	—	—
L.V 6	85	—	— A	
L.VI 1	43 A			
L.VI 2	—	—	—	
L.VI 3	—	—	24	
L.VI 4	101 A			
L.VI 5	—	— A		
L.VI 6	—	—	—	
L.VII 1	2	1	9	
L.VII 2	—	1	—	
L.VII 3	—	—	—	
L.VII 4	103 A	—		
L.VII 5	—	— A		
L.VII 6	—	— A		
L.VIII 1	2	1	3	1
L.VIII 2	—	—	—	1
L.VIII 3	—	—	—	2
L.VIII 4	98	76 A		
L.VIII 5	—	—	—	73
L.VIII 6	—	—	—	82
L.IX 1	9	1	3	—
L.IX 2	—	—	—	10
L.IX 3	—	—	— A	
L.IX 4	101	98	63	102
L.IX 5	—	—	—	106
L.IX 6	—	— A		
B.I 1	6	4	3	2
B.I 2	1	5	3	2
B.I 3	0	2	7	2
B.I 4	98	90	96	60 A
B.I 5	93	85 A		
B.I 6	95	103	94	96
B.II 1	2	1	2	3
B.II 2	9	1	1	4 A
B.II 3	2	2	2	2
B.II 4	98	13	30	62
B.II 5	99	95	97	102
B.II 6	97	41	65	83 A

Note.—In all seven groups, Nos. 1, 2, and 3 were given anti-D and Nos. 4, 5, and 6 were controls.

Experiment III (Further Clearance Studies)

The results are given in Table VI. It can be seen that the injected cells were most rapidly cleared when the plasma contained predominantly incomplete antibodies.

TABLE VI.—*Results of the ^{51}Cr Studies and Whether or Not Immune Antibodies (A) Had Developed Before the Next Stimulus in Experiment III*

Group and Volunteer Nos.	Percentage of Rh-positive Cells Surviving 24 Hours after Injection			
	1st Stimulus	2nd Stimulus	3rd Stimulus	4th Stimulus
B.III 1	3	0	7	4
B.III 2	1	0	15	4
B.III 3	1	0	4	3
B.III 4	47 A			
B.III 5	11	16	26	3 A
B.III 6	55	38	54	46 A
B.IV 1	5	1	29 A	
B.IV 2	1	1	20	1
B.IV 3	1	1	10	2
B.IV 4	31	4	75 A	
B.IV 5	34	5	73	4
B.IV 6	26 A			
B.V 1	89	86	60	66
B.V 2	101	86	—	—
B.V 3	94	85	100	94
B.V 4	89	101	94	97
B.V 5	96	36	61 A	
B.V 6	89 A			
B.VI 1	8			
B.VI 2	3	1	2	
B.VI 3	11	2	3	
B.VI 4	26	85	87	
B.VI 5	106			
B.VI 6	110	94	102	
B.VII 1	46 A	—		
B.VII 2	62 A	—		
B.VII 3	32	11 A		
B.VII 4	92 A	—		
B.VII 5	92	108 A		
B.VII 6	98	103		

Note.—Details of anti-D dosage are given in Table III.

Fate of Cleared Erythrocytes

In the case of L.V. second stimulus, detailed studies of the clearance rate for the three treated individuals during the first 24 hours were carried out. An antibody showing no saline activity and an albumin titre of 8 was used. Counts of radioactivity were taken at the same time over the splenic area, and Table VII shows the results obtained. A concentration of radioactivity was found in this area, and in volunteer No. 3 it was remarkably high. We are not sure of the explanation of this, but it may be relevant that, although he was entirely symptomless, his serum bilirubin was 1.5 mg./

TABLE VII.—*Results of Studies of Splenic Radioactivity in Volunteers 1, 2, and 3 of Group L.V. at Time of Their Second Injection of 5 ml. Rh-positive Blood Followed by 50 ml. of Anti-D. Results of Heart and Spleen Counts are Expressed as Percentages of Heart Counts One Hour After Injection of Anti-D. Results are Also Given of Counts of Venous Blood Samples Taken at Same Time, These Being Expressed as a Percentage of the Venous Sample Count at Time of Anti-D Injection*

Time after Anti-D Injection	Organ	Volunteer No.		
		1	2	3
−1 minute	Blood	100	100	100
1 hour	Blood	92	100	83
	Heart	100	100	100
	Spleen	37	25	374
2½ hours	Blood	84	75	36
	Heart	100	53	55
	Spleen	26	23	498
4 ,,	Blood	61	50	15
	Heart	135	71	49
	Spleen	56	43	769
5½ ,,	Blood	44	33	8
	Heart	78	63	69
	Spleen	41	39	795
24 ,,	Blood	2	1	0

100 ml. at that time. However, the spleen findings in general tend to support the view that coated cells are principally removed from the circulation by the spleen (see Jandl *et al.*, 1957 ; Mollison, 1959).

Discussion

The main findings in the experiments described were: (1) 10–20 ml. of plasma containing mainly complete anti-D failed to clear rapidly Rh-positive erythrocytes from the blood of Rh-negative male subjects and enhanced the immunization produced by these cells ; and (2) 35–50 ml. of plasma containing chiefly incomplete anti-D usually produced rapid clearing of Rh-positive red cells and considerably suppressed immunization by these cells in Rh-negative male subjects.

The relationship between the various factors may be considered under the following headings: (*a*) effectiveness of various anti-D antibodies in clearing injected Rh-positive cells from the circulation ; (*b*) relationship between the clearance rate of such cells and subsequent immunization ; and (*c*) relationship between differing amounts and types of anti-D antibody and subsequent immunization.

Antibody and Clearance.—Considering only the men treated with 35–50 ml. of plasma, it can be seen that in general when the plasma contained more than a trace of complete antibody only partial clearance was obtained in the first 24 hours. Rapid clearance was occasionally brought about by quite low-titre plasmas if they had no complete component—for example, L.V, first and second stimuli, and B.IV, first stimulus—but where there was more than a trace of saline activity a very high titre of incomplete antibody was needed to obtain satisfactory clearance—for example, B.III, first stimulus. Where the saline titre was more than 16, a good clearance was not found even if the incomplete titre was very high—for example, B.IV, third stimulus, and B.V, third and fourth stimuli.

Clearance of Injected Red Cells and Immunization.—Only those subjects in whom ^{51}Cr-labelled cells were followed for survival can give any information on this point. On examining the data in Tables IV, V, and VI it is obvious that immune antibody did not appear in most of the men in whom good clearance of the Rh-positive cells was obtained each time they were injected. Conversely, immunization occurred in many more of the men in whom only partial clearance was achieved than in those men in whom the cells were allowed to survive normally. This is shown in Table VIII. The data suggest that immunization is nearly always prevented in those volunteers in whom 95% of the injected cells have been cleared within 24 hours. One individual (B.II 2) developed antibodies in spite of good clearance rates on four successive occasions, but it might be important that 9% of the injected cells of the first stimulus survived 24 hours.

TABLE VIII.—*Relationship Between Speed of Removal of 5 ml. of Injected Rh-positive Cells from Circulation and Whether or Not Immune Antibodies Developed*

R.B.C. Survival Score†	First Stimulus Only			Four Stimuli*		
	No. of Men	Developing Antibodies		No. of Men	Developing Antibodies	
		No.	%		No.	%
0–10	18	0	0	11	1	9·1
10–75	17	7	41·2	11	8	72·7
75–100	32	5	15·6	8	3	37·5

* An R.B.C. survival score has been calculated for each man in groups B.I.–B.V. by averaging the percentages of red cells remaining in the circulation 24 hours after each stimulus.

† Survival scores were grouped into the three categories after examination of the data had shown that the critical 24-hour level for protection might be 10%.

Immunization and Amount and Type of Antibody Used.—From a consideration of all the Tables it can be seen that when plasma containing a high titre of complete antibody was used several of the men became immunized (group L.I–III). When 10-ml. volumes were used, even though the anti-D was predominantly incomplete but had some complete component, immunization often occurred (B.III–V). In the case of B.IV 1, who received at the third stimulus 50 ml. of plasma with a high titre of incomplete antibody but also showing considerable saline activity, the injection was followed by antibody formation. Only where the anti-D given had no activity in saline and was given in volumes of 35–50 ml. did most of the men obtain protection against immunization. As might be expected, the titre of such an antibody appeared to influence the result—for example, the use of 50 ml. of plasma with a weak incomplete anti-D in L.VI at the first stimulus was followed by the development of antibodies in one of the three men.

From the analysis of the data we interpret: (1) That any complete antibody in the plasma used is likely to enhance rather than prevent sensitization. (2) That plasma containing only incomplete antibody if given in volumes of 35–50 ml. will usually prevent immunization. Although there is not a clear relationship between the titre of such an antibody and immunization it would appear safer to use the highest titre antibody possible if immunization is regularly to be suppressed.

Some Immunological Problems

Red-cell Survival in Controls.—In most cases the first injection of Rh-positive cells into our controls was followed by normal survival of the cells with gradual clearance over a period of three months. Subsequent injections also had, in general, normal survival if no immune antibodies had developed. But in common with other workers (see Mollison, 1959) we occasionally found reduced survival time without demonstrable antibody. Two non-identical twins (B.V 5 and 6) had normal survival times with the first stimulus, and immune anti-D was subsequently detected in B.V 6 six weeks later. At the second stimulus both twins showed a marked and equal reduction in the survival time of the donor cells, but at the end of a further six weeks immune anti-D could not be detected in B.V 5 either by ourselves or by two other laboratories. A third stimulus was given and B.V 5 again had a reduced survival time, and on retesting six weeks later immune anti-D was detected for the first time. A similar situation occurred in B.II 4 and 6, in whom reduced survival times were demonstrated with their second, third, and fourth stimuli, again suggesting immunization. Immune antibody was finally demonstrated in B.II 6 five months after the fourth stimulus, but not in B.II 4. If this immunological state (reduced red-cell survival time without demonstrable antibody) had occurred in our "treated" series we should not have detected it, as their ^{51}Cr studies were always affected by the anti-D serum they had received. To safeguard against the late development of antibody (as in B.II 6) all subjects were retested about five months after their last stimulus.

Mechanisms of Protection and Enhancement.—The data presented are compatible with the hypothesis that

incomplete anti-D prevents immunization by coating the red-cell surface and thus blocking the Rh antigen sites (Stern *et al.*, 1961). The protection afforded by ABO incompatibility clearly cannot be due to the same mechanism, and Stern *et al.* (1961) have suggested that this may be due to a "clonal competition for antigen."

The enhancement of immunization by saline antibodies is more difficult to explain, although similar phenomena have been observed in other animal systems (see Cohen and Allton, 1962). We suggest as tentative hypotheses either that cells coated with saline antibody are dealt with at a different site in the reticulo-endothelial system at which, perhaps, immunization is more liable to occur; or that the layer of complete antibody is more easily removed from the red-cell surface. In the latter case the saline anti-D would simply attract the Rh-positive cells to the reticulo-endothelial system, and then break off, leaving large numbers of partially coated cells in contact with immunologically competent cells.

Prevention of Rh Haemolytic Disease

Our previous studies (Finn *et al.*, 1961) suggested that Rh immunization was most likely to occur with "large" transplacental haemorrhages of foetal blood—that is, greater than 1 ml.—and bleeds of this size occur more commonly during delivery. On the other hand, we have observed occasional bleeds of the same order of magnitude to occur during pregnancy, and these probably account for a certain percentage of cases of primary immunization. There is also evidence (Kristoffersen *et al.*, 1962) that minute bleeds are not uncommon during the course of pregnancy, and it remains to be seen whether these are capable of inducing antibody formation in susceptible individuals or whether there is a critical size of bleed below which immunization is unlikely. On the whole we think that the evidence points to the fact that the majority of bleeds sufficient to cause immunization *do* occur during labour, and operative trauma at this time has been shown to increase their incidence (Wimhöfer *et al.*, 1962). If this be confirmed, then the present approach to the prevention of immunization by the use of incomplete Rh antibody could contribute considerably towards the prevention of Rh immunization, the antibody being given to an Rh-negative mother after the delivery of an Rh-positive child if foetal cells are demonstrated in her circulation.

Further Work Proposed

We are not entirely happy about giving whole plasma or serum, since it is not without risk of producing hepatitis. This difficulty can be overcome by using a concentrated gamma-globulin preparation containing only incomplete anti-D antibodies. We tested such a preparation and found that 5 ml. given intramuscularly was much more powerful in producing clearance than 50 ml. of high-titre antiserum given intravenously. As well as ease of administration and freedom from the risk of producing serum hepatitis, this technique has the advantage of reducing to negligible proportions the risk of enhancing immunization, because in the preparation of the gamma-globulin an adequate titre and lack of saline activity can be ensured.

Another aspect to be investigated is the delaying of the anti-D injection up to 48 hours after the Rh-positive blood, to see if immunization can still be prevented; this is particularly important, since it is the situation which is apt to occur in hospital practice.

So far our experiments have only been in Rh-negative men using adult Rh-positive blood. We feel that it is essential now to find out if foetal blood can be cleared equally well and also to use sterile female volunteers, preferably pre-menopausal. If this is as successful as in the men we think that the evidence will be strong enough to try the technique on Rh-negative women who are having their first Rh-positive baby and in whom transplacental haemorrhage has occurred.

Summary

The results are described of experiments involving the injection of Rh-positive blood into 96 Rh-negative men and designed to find out whether or not the production of immune anti-D can be prevented.

Giving 10–20 ml. of anti-D sera containing high titres of complete antibody half an hour after the Rh-positive blood, we found that only about 50% of the injected cells had been cleared within 48 hours and immune anti-D production was enhanced as compared with controls, who received only the Rh-positive blood.

Using 35–50 ml. of plasma containing predominantly incomplete antibodies, we found that only 3 out of 21 "treated" men developed immune antibodies after three or four stimuli as compared with 11 out of 21 control men, the difference being statistically significant ($P=0.02$).

Examination of these results and those of other experiments which are described suggests that about 95% of the injected cells have to be cleared from the circulation within 24 hours if immune antibody production is to be prevented. The anti-D antibody most likely to be effective in this should have no saline activity and as high an incomplete titre as possible.

Preliminary work with anti-D gamma-globulin given intramuscularly has shown that in appropriate dosage it is even more effective in rapidly clearing Rh-positive cells than the most powerful plasma we have used.

Before the stage is reached at which a clinical application of the technique in recently delivered Rh-negative women is justifiable, additional experiments are indicated and these are outlined. We are hopeful, however, that the technique will prevent most cases of Rh immunization and thus in time help to eliminate Rh haemolytic disease of the newborn.

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REFERENCES

Cohen, C., and Allton, W. H. (1962). *Nature (Lond.)*, **193**, 990.
Finn, R., Clarke, C. A., Donohoe, W. T. A., McConnell, R. B., Sheppard, P. M., Lehane, D., and Kulke, W. (1961). *Brit. med. J.*, **1**, 1486.
—— —— McConnell, R. B., Woodrow, J. C., Kulke, W., Lehane, D., and Sheppard, P. M. (1962). *Nature (Lond.)*, **193**, 990.
Jandl, J. H., Jones, R. A., and Castle, W. B. (1957). *J. clin. Invest.*, **36**, 1428.
Kristoffersen, K., Jensen, K. G., and Felbo, M. (1962). *Dan. med. Bull.*, **9**, 203.
Levine, P. (1943). *J. Hered.*, **34**, 71.
Mollison, P. L. (1959). *Brit. med. J.*, **2**, 1035, 1123.
Race, R. R., and Sanger, R. (1950). *Blood Groups in Man*, 1st ed., p. 290. Blackwell, Oxford.
Stern, K., Goodman, H. S., and Berger, M. (1961). *J. Immunol.*, **87**, 189.
Wimhöfer, H. von, Schneider, J., and Leidenberger, F. (1962). *Geburtsh. u. Frauenheilk.*, **22**, 589.

TREATMENT OF TUMOURS OF THE TESTIS

282 TESTICULAR TUMOURS SEEN AT THE LONDON HOSPITAL DURING 1926–61

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Reappraisal of Lymphadenectomy

The late Sir Hugh Cairns reported in 1926 a series of 95 testicular tumours seen at the London Hospital during 1908 to 1925: 55 of these were treated by simple and 19 by radical orchidectomy. After carefully analysing his results Cairns wrote: "I am of the opinion that the results are slightly in favour of the radical operation. Yet it is questionable whether they are a sufficient improvement over the results of orchidectomy to justify the added risk. . . ." As for the use of radiotherapy, then in its infancy, Cairns dismissed it briefly: "In this series treatment of metastases by *x* rays has been entirely unsuccessful. Some workers have reported good results from the use of radium, but of this I have had no experience."

The next two decades were to see a major change in the approach to treatment. As the techniques of radiotherapy improved so did the results, until by the end of the second world war British surgeons no longer felt that there was any legitimate indication for the radical operation (Gordon-Taylor and Wyndham, 1947).

Since then, for the past fifteen years, simple orchidectomy followed by radiotherapy has been the standard treatment for any testicular tumour, at least in the United Kingdom (Boden and Gibb, 1951; Whittle, 1957; Stephen, 1958; Prossor, 1959; Badenoch and Pugh, 1961).

To-day this position is under attack. In North America, where the radical operation never went completely out of favour, a plausible case has been made out for the surgical removal of abdominal lymph nodes, in addition to radiotherapy, for those types of teratoma which are regarded as being particularly radio-resistant. At first view the published results of adding lymphadenectomy to a course of radiotherapy appear better than any obtained with radiotherapy alone (Patton, Seitzman, and Zone, 1960).

The operation itself has been improved. New techniques of lymphadenectomy, which go further to satisfy the exacting requirements for complete removal of testicular lymphatics (Jamieson and Dobson, 1910), have now been carried out with an acceptably low mortality (Cooper, Leadbetter, and Chute, 1950; Cahill, 1961; Lewis, 1953; Leadbetter, 1953; Staubitz, Magoss, Oberkircher, Lent, Mitchell, and Murphy, 1958; Dowd, Chute, and Weinert, 1959; Tobenkin, Binkley, and Smith, 1961; Mallis and Patton, 1958).

The present investigation began with the object of finding out whether the omission of lymphadenectomy had resulted in a significantly poorer survival rate for teratomata. Was there a case for changing the present policy of treatment?

No comparative series had ever been treated by radiotherapy with and without lymphadenectomy, so that there existed no clear-cut answer to this question, and without it the same ethical considerations which prevented one surgeon from withholding lymphadenectomy prevented another from performing it.

One commendable attempt to evaluate these alternative methods of treatment was made by Staubitz *et al.* (1958), who compared a series treated during 1922–48 with another series treated in 1949–53. Their study had the merit of dealing with cases seen in a single centre, but the numbers in the second group were small, and the comparison took no account of the advances made in all spheres of treatment, including radiotherapy, over a period of thirty years. Lymphadenectomy had been performed in only 16 patients, and in only six of them were metastases present in the operation specimen —evidence which scarcely justified the conclusion that the slight improvement in the survival rate for teratoma was to be attributed to lymphadenectomy.

The comparison of results from one centre with those from another is open to so many sources of error that authorities have condemned it out of hand when used, for example, in the study of carcinoma of the breast (Atkins, 1960). But in the face of the new claims for lymphadenectomy some attempt must be made to evaluate them, even if not all the evidence is above criticism.

One possible error arises out of the nature of the treatments to be compared. Lymphadenectomy provides the surgeon with histological proof of abdominal metastases. These will be found at operation in approximately one-third of all patients in whom nodes are impalpable before operation (Cahill, 1951; Patton *et al.*, 1960). In a proportion of these cases the unsuspected nodes will be fixed and inoperable, a proportion which varies between some 37 and 16% of cases explored (Dowd *et al.*, 1959; Patton *et al.*, 1960). These advanced cases will be excluded from the results of a series treated by lymphadenectomy, but included in a series treated by radiotherapy alone.

A different type of error arises from variations in pathological classification. In the United Kingdom testicular tumours are usually classified into mixed tumours, or teratomata, and seminomata (Cairns, 1926; Gordon-Taylor and Wyndham, 1947; Snelling and