a co-operative study in many areas and given here are interim and do not necessarily represent final conclusions. On the proportion of those immune to rubella because of past infection this has averaged 89% in our survey of 2,007 sera, ranging between 80 and 96% in different parts of the country. In the quite separate investigation of 1,747 pregnant contacts of rubella, serum from whom was obtained before immunoglobulin was given, 85% had immunity. These figures agree generally with previous reports from this and other countries and are applicable whether or not there has been a past history of rubella. In any event, as shown separately (P.H.L.S., 1968) immunoglobulin in susceptible volunteers has only a marginal effect in terms of circulating antibody. Because the risk of a second attack of rubella appears slight it would seem that most of the contacts received immunoglobulin unnecessarily. With the serological tests available it should now be practicable to test for rubella antibody serum from any woman before or as soon as possible after the start of pregnancy.

Previous estimates of the protective effect of immunoglobulin against rubella have been obscured by the high proportion already immune. Protection can only be accurately assessed in the small proportion who are susceptible. On this one criterion, irrespective of whether the illness in the index case was always true rubella, 46 out of 264 (17%) of those in our inquiry given the standard amount of globulin developed serologically proved rubella. With the further restriction of confirmation of rubella in the index case by virus isolation the number of susceptible persons fell to 25 and 14 of these developed serologically proved rubella. It was also found that in 12 of the 14, the index case was a member of the same household, a pointer to the high risk from close contact. The same criterion of virus isolation from the index case in the, at present, small control group of household contacts not pregnant and not given immunoglobulin showed that seven out of nine susceptible contacts developed the disease, again pointing to the high risk from close contact.

When only susceptible women in contact with proved rubella are considered the figures admittedly are small, but they do not suggest any protective effect against rubella by immunoglobulin in the dosage used. Larger amounts already tried (P.H.L.S., 1967) seem to have been no more effective, but the problem is to select and treat rapidly those specially at risk. Further studies will be done, but it is probable that ultimately an active vaccination programme will be more successful. From the findings rubella is as likely to be inapparent as it is to be an illness with a rash, so that it is difficult to decide on clinical grounds whether immunoglobulin has any suppressive effect on the illness. It did not do so in the one second attack recorded.

Because of the opportunities for therapeutic abortion and other factors follow-up information on infants born of mothers who had rubella during pregnancy is not at present available for this series.

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REFERENCES

Beale, A. J., Christofinis, G. C., and Furminger, I. G. S. (1963). *Lancet*, 2, 640.
Brit. med. J., 1967, 4, 183.
Field, A. M. (1967). *J. Hyg. (Lond.)*, 65, 409.
Field, A. M., Vandervelde, E. M., Thompson, K. M., and Hutchinson, D. N. (1967). *Lancet*, 2, 182.
Hutchinson, D. N., and Thompson, K. M. (1965). *Mth. Bull. Minist. Hlth Lab. Serv.*, 24, 385.
McDonald, J. C. (1963). *Brit. med. J.*, 2, 416.
McDonald, J. C., and Peckham, C. S. (1967). *Brit. med. J.*, 3, 633.
Public Health Laboratory Service (1967). *Brit. med. J.*, 3, 638.
Public Health Laboratory Service (1968). *Brit. med. J.*, 3, 206.

Measurement of Rubella Antibody in Immunoglobulin: its Disappearance from the Blood after Injection

Report of the Public Health Laboratory Service Working Party on Rubella*

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Summary: Rubella antibody titrations were done on samples of human immunoglobulin by neutralization and haemagglutination-inhibition methods. No significant variation was found in the antibody content of different batches. The specificity of the methods was confirmed by tests on a batch of human globulin specially prepared from plasma samples lacking rubella antibody.

Divided doses of immunoglobulin were given to volunteers who had no rubella antibody. Low titres were then detected in the blood for a limited period and the disappearance of this antibody was followed.

Introduction

Because of the chance of congenital abnormality after maternal rubella in early pregnancy human immunoglobulin is given prophylactically to pregnant women at risk.

Recently it has become possible to estimate the rubella antibody in human immunoglobulin, but titres appear to vary widely. Generally the higher range of titres, 1/256 to 1/2,048, were obtained with low virus dosage, 25 TCD_{50} or less, in neutralization tests (Schiff, Sever, and Huebner, 1963; Neva and Weller, 1964; Oxford, 1966; Murphy and Reid, 1967). Lower antibody titres, 1/64 to 1/512, were reported when a virus dose of 100 TCD_{50} was used (Parkman, Mundon, McCown, and Buescher, 1964; Givan, Rozee, and Rhodes, 1965; Green, Balsamo, Giles, Krugman, and Mirick, 1965; Picciotto, 1965; Hull and Butorac, 1966). Stewart, Parkman, Hopps, Douglas, Hamilton, and Meyer (1967) found rubella haemagglutination-inhibiting antibody titres of immunoglobulin to be in the range 1/1,024 to 1/4,096.

In the investigation now reported the rubella antibody content of a number of batches of human immunoglobulin used for rubella prophylaxis was assayed by neutralization and haemagglutination-inhibition methods. Specificity of the tests was checked by means of a specially prepared batch of human globulin devoid of rubella antibody. The decline and disappear-

* Report prepared by Dr. Anne M. Field and Dr. Elise M. Vandervelde.

ance of measurable antibody in susceptible volunteers injected with immunoglobulin was also studied.

Materials and Methods

Immunoglobulin.—The human immunoglobulin tested was prepared at the Lister Institute. It had been stored at 4° C.

Rubella Neutralization Tests.—Neutralization tests were done in R.K.$_{13}$ cells by the method described (Field, 1967) and also in vervet monkey kidney cell cultures by the challenge method of Parkman, Buescher, and Artenstein (1962). In this latter test the preliminary mode of inoculation was similar to the method for the R.K.$_{13}$ cells, but after seven days a challenge dose of 100 TCD_{50} E.C.H.O. 11 virus was added. The tubes were re-examined at intervals over the next five days for cytopathic change. A lack of this indicated the presence of rubella virus. The human sera were tested for neutralizing antibodies in R.K.$_{13}$ cells only. The human globulin samples were tested by both methods. Neither the sera nor the globulin samples were heated at 56° C. before testing. Antibody titres were expressed as the highest initial dilutions which neutralized rubella virus in 50% of the tubes.

Rubella Haemagglutination-inhibition Tests.—The previously described micromethod was used (Field, Vandervelde, Thompson, and Hutchinson, 1967). Sera and immunoglobulin samples were treated with kaolin, adsorbed with chick red blood cells, and inactivated before testing. Antibody titres were expressed as the highest dilutions which inhibited agglutination of 1-day-old chick cells by rubella haemagglutinin.

Results

Neutralizing Antibody in Samples of Human Immunoglobulin.—Immunoglobulin was found to be moderately toxic to R.K.$_{13}$ cells in culture and very toxic to vervet monkey kidney cells. The cytotoxicity endpoint for R.K.$_{13}$ cells occurred most often between the 1/10 and 1/20 dilutions, but toxic effects were seen regularly at the 1/20 and 1/40 dilutions in the vervet monkey kidney cell cultures. Great difficulties were experienced in obtaining a definite rubella antibody endpoint for immunoglobulin by the R.K.$_{13}$ cell method, though clear-cut endpoints were always found in these cells when titrating antibody in human sera. Evidence of considerable virus multiplication always occurred in the immunoglobulin titrations towards the end of the standard period of incubation of the cultures. If antibody titres were expressed as the highest dilutions completely inhibiting rubella virus cytopathic effect the titres were low, not greatly in excess of those found in human sera, and were close to the cytotoxicity endpoints. Because of this, traces of cytopathic effect in the R.K.$_{13}$ cell test were ignored and titres of antibody in immunoglobulin were expressed as the highest dilution which substantially neutralized the virus. In practice this neutralization always represented at least a 90% reduction in the number of rubella microfoci. The neutralizing antibody titres of immunoglobulin found in vervet monkey kidney cell tests were similar to those in R.K.$_{13}$ cell tests (Table I). There was no marked variation among the different samples tested. Titres ranged from 1/40 to 1/320, the higher titres occurring when lower virus doses were used. Antibody titres were approximately five times as high as those found in human sera.

TABLE I.—*Rubella Neutralizing Antibody in Samples of Human Immunoglobulin*

Sample Identification No.	Neutralizing Antibody Reciprocal Titres in:	
	R.K.$_{13}$ Cells	Vervet M.K. Cells
1	240 (30)	N.T.
2	160 (300)	N.T.
3	240 (30)	N.T.
4	180 (30)	N.T.
5a	80 (30)	N.T.
5b	320 (30)	N.T.
6	120 (30)	N.T.
7	80 (30)	N.T.
8	240 (30)	N.T.
9	120 (30)	N.T.
10a	240 (30)	120 (30)
10b	120 (30)	80 (100)
10c	240 (30)	120 (300)
10d	320 (30)	120 (300)
10e	80 (100)	—
10f	240 (100)	—
11	180 (30)	N.T.
12	180 (30)	N.T.
13a	60 (30)	80 (100)
13b	120 (30)	—
14	80 (300)	120 (100)
15	60 (300)	< 80 (100)
16	60 (300)	120 (100)
17a	40 (100)	120 (100)
17b	120 (100)	—
18	120 (100)	240 (30)

Figures in parentheses refer to TCD_{50} in R.K. cells and tissue culture interfering dose ($TCInD_{50}$) in vervet M.K. cells.
N.T. = Not tested. Letters a-f are repeat tests on the same sample.

Haemagglutination-inhibiting Antibody in Samples of Human Immunoglobulin.—Titres ranged between 1/1,600 and 1/12,800, higher titres being found when 4 units of haemagglutinin and lower titres when 8 units of haemagglutinin were used. Again there was no great variation between the titres of different batches (Table II). Titres were approximately 10 times those encountered in normal human sera.

TABLE II.—*Rubella Haemagglutination-inhibiting Antibody in Samples of Human Immunoglobulin*

Sample Identification No.	H.I. Antibody Titre	Sample Identification No.	H.I. Antibody Titre
1	1,600 (8)*	18	3,200 (8)
2	1,600 (8)	19	12,800 (4)
3	1,600 (8)	20	12,800 (4)
5	1,600 (8)	21	6,400 (4)
10	3,200 (8)	22	6,400 (4)
	6,400 (8)	23	12,800 (4)
13	1,600 (8)	24	6,400 (4)
15	1,600 (8)	25	12,800 (4)
16	1,600 (8)	26	12,800 (4)

* Units of rubella haemagglutinin used in the inhibition test.

Specificity of Antibody Tests on Immunoglobulin.—All the ordinary batches of immunoglobulin tested contained rubella antibodies. These are prepared from pools of human plasma, and, because most blood donors are likely to have some rubella antibody, this result is to be expected. In order to see whether the tests specifically measure rubella antibodies in human immunoglobulin a special batch of human globulin was prepared at the Lister Institute Blood Products Division from a pool of four samples of human plasma. These were selected because they lacked rubella antibody in neutralization and haemagglutination-inhibition tests. This special batch and, as a control, an ordinary batch of human globulin were made available with and without the addition of 1/10,000 thiomersal (Merthiolate) as preservative, this being the element responsible for cytotoxicity. This specially prepared human globulin was, as expected, negative in all antibody tests (Table III). When thiomersal was added there was, however, a considerable reduction of rubella cytopathic effect at the 1/10 and 1/20 dilutions as shown by microfocus counts (Table IV). The sample of ordinary human globulin with thiomersal gave results comparable with all other batches tested. The sample lacking thiomersal, however, showed less than expected neutralization of rubella virus in R.K.$_{13}$ cells (Table III).

TABLE III.—*Specificity of Rubella Antibody Tests*

Test	Rubella Antibody-free Globulin		Globulin Sample No. 18	
	With Thiomersal	Without Thiomersal	With Thiomersal	Without Thiomersal
Neutralization:				
In R.K.$_{13}$ cells ..	< 1/20*	< 1/10	1/120	< 1/40
In vervet M.K. cells	< 1/120*	< 1/8	N.T.	1/240
Haemagglutination-inhibition	< 1/16	< 1/16	1/3,200	1/3,200

* Dilutions below 1/20 were toxic for R.K.$_{13}$ cells and below 1/120 were toxic for vervet monkey kidney cells.
N.T. = Not tested.

TABLE IV.—*Effect of Thiomersal in Rubella Antibody-free Human Globulin on Rubella Virus Cytopathic Effects*

Inoculum	No. of Rubella Microfoci in Duplicate Tubes	
	1	2
Antigen control	240	230
Globulin without thiomersal diluted 1/10	203	224
Globulin with thiomersal — Diluted 1/10	19	45
Globulin with thiomersal — Diluted 1/20	135	96

Rubella Antibodies in the Blood After Injection of 750 mg. of Immunoglobulin.—In the course of the inquiry into its effectiveness sera were collected from pregnant women at various times after the injection of 750 mg. of immunoglobulin because of contact with suspected rubella (P.H.L.S., 1968). From 12 of these, all lacking rubella antibody, sera were collected between 4 and 12 weeks after the injection with immunoglobulin. The tests for neutralizing and haemagglutination-inhibiting antibodies were quite negative. In two further instances sera taken 1 and 13 days respectively after injection were also completely negative.

Rubella Antibodies in the Blood After Injection of 3,000 mg. of Immunoglobulin.—Five young adult women without rubella antibodies were bled and injected with 1,500 mg. of immunoglobulin. Two days later (in one instance four days later) a further blood sample was taken and the immunoglobulin inoculation was repeated. Further blood samples were taken at intervals (Table V). The sera were titrated for rubella neutralizing and haemagglutination-inhibiting antibodies. Low antibody titres, detectable in both tests, appeared in the blood after the first dose of immunoglobulin and were maintained virtually unchanged for three weeks. Some reduction of titre was observed by the fourth week and antibody had disappeared 12 weeks after inoculation.

TABLE V.—*Rubella Antibody After Injection of Volunteers with 3,000 mg. of Immunoglobulin*

Volunteer	Day	Rubella Antibody Titres			
		Neut.		H.I.	
		100 TCD_{50}	20 TCD_{50}	2 H.A. Units	8 H.A. Units
1	0*	<2	<2	<4	<4
	2*	2	4	8	<16
	4	2	6	32	8
	14	3	4	64	N.T.
	25	<2	<2	16	<64
2	0*	<2	3	<4	<4
	2*	<2	4	16	4
	4	2	6	32	16
	15	4	8	64	8
	26	3	6	64	<8
3	0*	<2	N.T.	<4	<4
	4*	8	N.T.	16	8
	6	8	N.T.	32	8
	18	12	N.T.	32	8
	32	12	N.T.	16	8
4	0*	<1	N.T.	N.T.	<4
	2*	2	N.T.	N.T.	8
	4	3	N.T.	N.T.	16
	13	2·5	N.T.	N.T.	16
	23	3	N.T.	N.T.	16
	88	1·5	N.T.	N.T.	<4
5	0*	1	N.T.	N.T.	<4
	2*	1·5	N.T.	N.T.	<4
	4	3	N.T.	N.T.	16
	13	3	N.T.	N.T.	16
	23	3	N.T.	N.T.	8
	88	1·5	N.T.	N.T.	<4

* Days on which 1,500 mg. immunoglobulin was given.
N.T. = Not tested.

Discussion

For some 14 years human immunoglobulin or gammaglobulin has been used in the prophylaxis of rubella in this country, and McDonald and Peckham (1967) have put forward evidence in favour of its usefulness. More recently it has become possible to measure the rubella antibody content in human serum and the globulin derived from it. Both neutralization and haemagglutination-inhibition tests have been used. From evidence already presented more than 80% of adults in this country appear to have circulating rubella antibody. The globulin is prepared from pools of adult plasma. It was not surprising from the tests (Tables I and II) on a number of batches to find that all contained rubella antibody. By previous selection of negative plasma samples, however, it was possible to obtain a special batch without antibody for checking the specificity of the tests.

Problems encountered in the neutralization tests are reported, including the difficulty of getting sharp endpoints. In part this may be due to the absence of the labile factor present in unheated sera and in part it may be affected by the cytotoxicity of the added preservative (thiomersal). Possible reasons for the considerable variation in titres in published reports are also included.

The use of immunoglobulin for rubella prophylaxis has been followed by the temporary appearance of low-titre circulating antibody. At the standard dosage of globulin this has not been shown, but the tests are not necessarily very sensitive and circumstances did not favour the collection of repeated blood samples from pregnant women at ideal time intervals. It is obviously not necessary to give immunoglobulin to the majority of those exposed to rubella, because they are already immune. The crucial question which remains is whether larger amounts than 750 mg. given as early as possible to those who are susceptible would have a more protective effect. At this stage active immunization against rubella of young adult women still susceptible seems a more promising alternative.

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REFERENCES

Field, A. M. (1967). *J. Hyg. (Lond.)*, **65**, 409.
Field, A. M., Vandervelde, E. M., Thompson, K. M., and Hutchinson, D. N. (1967). *Lancet*, **2**, 182.
Givan, K. F., Rozee, K. R., and Rhodes, A. J. (1965). *Canad. med. Ass. J.*, **92**, 126.
Green, R. H., Balsamo, M. R., Giles, J. P., Krugman, S., and Mirick, G. S. (1965). *Amer. J. Dis. Child.*, **110**, 348.
Hull, R. N., and Butorac, G. (1966). *Amer. J. Epidem.*, **83**, 509.
McDonald, J. C., and Peckham, C. S. (1967). *Brit. med. J.*, **3**, 633.
Murphy, A. M., and Reid, R. R. (1967). *Med. J. Aust.*, **1**, 369.
Neva, F. A., and Weller, T. H. (1964). *J. Immunol.*, **93**, 466.
Oxford, J. (1966). *Publ. Hlth (Lond.)*, **80**, 271.
Parkman, P. D., Buescher, E. L., and Artenstein, M. S. (1962). *Proc. Soc. exp. Biol. (N.Y.)*, **111**, 225.
Parkman, P. D., Mundon, F. K., McCown, J. M., and Buescher, E. L. (1964). *J. Immunol.*, **93**, 608.
Picciotto, L. (1965). *G. Mal. infett.*, **7**, 1013.
Public Health Laboratory Service (1968). *Brit. med. J.*, **3**,
Schiff, G. M., Sever, J. L., and Huebner, R. J. (1963). *Science*, **142**, 58.
Stewart, G. L., Parkman, P. D., Hopps, H. E., Douglas, R. D., Hamilton, J. P., and Meyer, H. M. (1967). *New Engl. J. Med.*, **276**, 554.