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Rabies Immune Globulin of Human Origin: Preparation and Dosage Determination in Non-Exposed Volunteer Subjects

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A clinical study was carried out in volunteer subjects to determine the proper dosage of rabies immune globulin (human) (RIGH) when used in conjunction with rabies vaccine of duck-embryo origin (DEV). Two lots of RIGH were prepared from plasma pools derived from donors with high titres of rabies-neutralizing antibody; both lots had potencies in excess of that accepted for antirabies serum of equine origin. The subjects had never received rabies vaccine but would ordinarily have been given pre-exposure rabies prophylaxis. Serological results obtained during a period of 90 days after the initiation of the study justify the following conclusions: (1) the half-life obtained with RIGH is in agreement with that estimated for homologous passive immune globulin in man; (2) readily detectable levels of rabies antibody were found in all subjects 24 hours after the administration of 40 and 20 international units per kg of RIGH, but not after the administration of 10 international units/kg; and (3) there was an indication that 40 international units/kg may have interfered with optimal active antibody production by the vaccine, and that interference was absent or minimal after doses of 20 international units/kg.

The use of rabies immune serum or globulin of equine origin, in combination with rabies vaccine, has been routine for many years in post-exposure immunization of man (WHO Expert Committee on Rabies, 1966).

The first recorded preparation of rabies immune serum was described by Babes & Lepp (1889). Between that time and the 1930s a number of reports were published on the use of rabies immune serum or serum concentrates in the prevention of experimental rabies in animals, or in the post-exposure treatment of man; a comprehensive review of these reports was made by Habel (1945). Results of the early experiments ranged from complete protection against a rabies challenge to no protection at all, but the experiments were not adequately controlled and the numbers of animals used were small. Later experiments in small animals and monkeys were better controlled and, together with results obtained in man, gave suggestive evidence of the value of rabies immune serum.

In a systematic study of rabies immune serum of rabbit origin in mice, guinea-pigs, and monkeys, Habel (1945) found that serum prophylaxis alone gave better protection in post-infection treatment than vaccine alone, and that protection was enhanced when serum treatment was combined with a course of vaccine administration.

These observations in animals were later confirmed and extended by Koprowski & Black (1954), Koprowski & Cox (1951), and Koprowski, Van der Scheer & Black (1950).

The usefulness of rabies immune serum in post-exposure treatment of man was dramatically demonstrated in a group of persons bitten by a rabid wolf in Iran (Baltazard et al., 1955; Habel & Koprowski, 1955), and was later confirmed in the USSR with rabies immune globulin (Selimov et al., 1959). In Iran, of 17 persons with severe head wounds, 5 received 2 injections of serum on the first and fifth

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days, and phenolized rabies vaccine for 21 days; 7 received 1 serum injection and a 21-day course of vaccine; 5 received only the course of vaccine. In addition, a 6-year-old boy suffering from a crushed parietal bone and torn dura mater was given 6 serum injections and vaccine for 21 days. Rabies antibody was detected as early as the second day in all those receiving serum and vaccine, and the antibody remained detectable throughout the observation period. In contrast, antibody became detectable only after 19 days in the vaccine-only group. Of the 18 subjects, 4 died of rabies—namely, 1 of the 7 who received 1 serum dose and vaccine, and 3 of the 5 who received vaccine alone. The most striking survival was that of the 6-year-old boy.

A series of studies co-ordinated by WHO was subsequently undertaken to determine the optimum conditions under which rabies immune serum of equine origin and rabies vaccine can be used in man (Atanasiu et al., 1956, 1957, 1961, 1967). These studies carried out in human adults not previously exposed to rabies and with no history of rabies vaccination indicated that serum can interfere with the active immunity induced by the vaccine. They further demonstrated that this interference can be overcome by booster doses of vaccine 10 and 20 days after the end of the usual 12–14-dose series. Habel (1966) has recently indicated that a more effective regimen consists of booster doses of vaccine at 20 days, 2 months, and 6 months after the initial series.

Preformed rabies antibody of equine origin was also found to be useful in the local treatment of bite wounds. In the studies on this mode of treatment, the antibody was applied in the form of immune serum or of liquid or powdered globulin (Dean, 1966; Dean, Baer & Thompson, 1963; Kaplan & Paccaud, 1963; Kaplan et al., 1962). The favourable results obtained in experimental animals culminated in a recommendation by the WHO Expert Committee on Rabies (1966) that the topical use of preformed rabies antibody should be considered in all exposures, particularly in severe exposures.

The utility in man of rabies immune serum of equine origin has not been demonstrated without penalty; a high incidence of serum sickness and the risk of anaphylaxis accompany its use. Only a preparation of human origin would prevent this side-effect from occurring in man, while preserving the benefit of preformed antibody. Hosty et al. (1959) and Anderson & Sgouris (1965) reported preliminary efforts to prepare rabies immune serum or globulin of human origin. More recently, Winkler, Schmidt & Sikes (1969) described the preparation of three lots of rabies immune globulin of human origin. Even though these lots were subpotent, they served to demonstrate the feasibility of this approach.

Homologous globulin preparations would have the obvious advantage of ending the risk of anaphylaxis or serum sickness. However, the question arises whether the longer-lasting homologous passive antibody might not interfere even more than equine globulin with the active immunity induced by the vaccine. Comparing the effect of equivalent dosages of homologous (guinea-pig) and heterologous (donkey) rabies immune sera in guinea-pigs, Archer & Dierks (1968) found that the former could delay or suppress active immunization when a rabies vaccine of avian origin was used. Delay or suppression did not take place, however, when a vaccine of higher potency was employed.

It is evident that additional studies are needed on the preparation of rabies immune globulin of human origin and on the conditions for its use in human immunization. This report summarizes our studies in this field.

MATERIALS AND METHODS

Rabies immune plasma: source, collection, and fractionation

Rabies immune plasma was obtained from healthy, volunteer adult donors who, because of actual past exposure to rabid animals, had received one or more courses of rabies vaccine (Semple, duck-embryo or chicken-embryo type) or who, because of the nature of their occupation (veterinarians, veterinary students, animal handlers), underwent routine pre-exposure immunization against rabies and received periodic boosters of rabies vaccine.

Sample bleedings from potential donors were first tested for rabies neutralizing antibody, and only those with titres considered high enough to yield an immune globulin preparation with an acceptable rabies antibody titre when the blood was pooled were asked to donate plasma. Blood was withdrawn by plasmapheresis in accredited blood banks at intervals not shorter than 1 week. The plasma units were stored frozen until they were tested individually for rabies antibody titre and were pooled for fractionation of the IgG.

Fractionation of pooled plasma was carried out by methods 6 and 9 of Cohn et al., (1946) and Oncley et al. (1949). The 16.5% ($\pm$2.5%) IgG solution obtained was sterile filtered and the final product was

aseptically dispensed in glass vials in 5-ml volumes. Biological and chemical control tests were carried out in accordance with US Public Health Service Regulations.[1] When all tests were completed, and found to meet the requirements set out in the Regulations, the assigned potency of the lot was printed on the label of the final container in terms of international units (IU) per ml.

Serum neutralization test

Individual plasma units, prefractionation plasma pools, and finished IgG preparations were tested for rabies neutralizing antibody according to the procedure recommended by the Division of Biologics Standards (DBS) of the US National Institutes of Health for the potency testing of antirabies serum (equine origin). Plasma samples, but not IgG samples, were inactivated at 56°C for 30 minutes before dilution.

The animals used were Swiss Webster mice of the same sex, weighing 10–14 g. The challenge virus was the CVS strain of rabies virus obtained from the Division of Biologics Standards. Stock virus was prepared from infected mouse brains and stored at −60°C or below in sealed glass ampoules.

One of the rabies immune preparations used as reference was DBS reference No. 2 and was in the form of a measured amount of dried globulin, which, when reconstituted with 5 ml of distilled water, was considered to contain 2 IU/ml of rabies antibody. The international unit is defined as the amount of antibody present in 4 mg of this particular dried reference globulin. The other preparation was a house reference serum made by us in rabbits.

The serum-neutralization test proper was carried out by preparing (1) equal parts of twofold dilutions of the house reference serum, each mixed with approximately 200 LD_{50}/0.03 ml of CVS virus, to monitor the reproducibility of the test system; (2) equal parts of twofold dilutions of the DBS Reference and CVS virus as above, to establish the denominator required for computation of the potency of the test plasma or globulin; (3) equal parts of twofold dilutions of test plasma or globulin and CVS virus; and (4) tenfold dilutions of the CVS virus suspension used in above mixtures to determine the number of mouse LD_{50}s used in each test.

The plasma- or globulin–virus mixtures were incubated at 37°C for 90 minutes and the mixtures were held in crushed ice until used. Each dilution was injected intracerebrally into 6 mice, each animal receiving 0.03 ml. The mice were observed for paralysis or death for 14 days, after which time virus or antibody titres were calculated by the Spearman–Kärber method (Spearman, 1908; Kärber, 1931).

In 34 consecutive tests carried out in this way, the number of virus LD_{50}s used in each varied between 22 and 220. The majority (21 tests) ranged from 47 to 100, the mean value was 91, and the median LD_{50} was 100. In the same 34 tests, the 50% serum-neutralizing dose (SN_{50}) of the house reference serum ranged from 1 : 89 to $\geqslant$1 : 316. The majority of SN_{50} titres (26 tests) were between 1 : 100 and 1 : 200, and their geometric mean was 1 : 163.

Clinical study

A study intended to investigate the optimum dose of rabies immune globulin (human) (RIGH), i.e., the dose that would result in minimal or no interference with the active immunity induced by rabies vaccine, was conducted at the Cowell Student Health Center and Hospital of the University of California. The study population consisted of consenting young adult volunteers from the School of Veterinary Medicine, aged 20–30 years, of either sex and in good health. Only individuals with no prior history of rabies immunization who would ordinarily receive pre-exposure rabies prophylaxis, were admitted to the study. They were randomly distributed among the first experimental groups to be described.

The RIGH employed in this investigation was experimental lot no. PR2316 (described below). This lot had a rabies antibody potency of 550 IU/ml. The vaccine used was a recently released lot (no. 3EE97B) of rabies vaccine, duck-embryo origin (DEV). It had a potency ratio of 1.17 against DBS reference vaccine no. 175 and, at the time it was administered, the expiration date was 12–13 months ahead.

EXPERIMENTS AND RESULTS

Lots of RIGH prepared

Following the procedures described above, two consecutive lots of RIGH, lot no. PR 2316 and lot no. PR 2342, were prepared. The first lot was derived from a pool of about 14 litres of plasma, and the second from 50 litres. Laboratory test results of the two lots are summarized in Table 1.

The potency of the two lots was determined both in the Cutter Laboratories and, through the courtesy of Dr Keith Sikes, in the Center for Disease Control.

[1] Title 42, Part 73, Biological Product, Revised June 1969.

Table 1. Cutter Laboratories test results on two experimental lots of rabies immune globulin (human) (RIGH)

Characteristic	Lot PR 2316	Lot PR 2342
date prepared	12 May 1969	11 February 1970
plasma pool:		
litres	14.25	50.0
IU/ml	22 [a]	10.6 [a]
RIGH:		
IU/ml	702 [a]–406 [b]	269 [a]–216 [b]
assigned IU/ml	550 [c]	240 [c]
pyrogen, sterility, identity tests	passed	passed
safety tests; mice and guinea pigs	passed	passed
protein concentration (%)	14.0	17.6
IgG purity (%)	98.1	98.9
mobility: cm^2/volt/s	1.21×10^{-5}	1.24×10^{-5}
pH	6.59	6.41
gelation after 4 hours at 57°C	none	none
turbidity after 7 days at 37°C	none	none

[a] Each value is a geometric mean of 3 determinations made at the Cutter Laboratories, Calif., USA.

[b] Single determination made at the Center for Disease Control, Atlanta, Ga., USA.

[c] Average values for RIGH from above-mentioned determinations.

The values obtained for the two RIGH lots were 22-fold to 85-fold higher than those of the plasma pooled; this ratio was in agreement with the concentration factor. The results obtained in the two laboratories were remarkably consistent, considering the limited precision of the assay procedure. The potency assigned to each preparation was the average of the values from the two laboratories, and was well above that usually obtained for antirabies serum (equine origin) in current use (Winkler, Schmidt & Sikes, 1969). This permitted substantially reduced volumes of RIGH to be administered to the volunteers admitted to the clinical study.

Determination of dosage

A total of 41 volunteer subjects participated in a study intended to determine the proper dosage of RIGH. They were divided into 5 groups, each of which received a different immunization schedule. The 5 schedules and the number of participants in each are presented in Table 2. Regardless of the dose of RIGH to be administered, lot PR 2316 was in all cases given only once—namely on the day the study was initiated. A chart of weights, ranging from 134 to 200 lb (61–91 kg) in 2–3-lb (1–1.4 kg) increments, was prepared in advance, the volumes of RIGH equivalent to 40, 20 or 10 IU/kilo being calculated.

Table 2. Immunization schedules of five groups of volunteer subjects with rabies immune globulin (human) (RIGH) *

	Group				
	A	B	C	D	E
no. of volunteers in group	8	8	8	9	8
RIGH dose (IU/kg [a]	40	none	40	20	10
DEV doses [b]	none	14 + 2	14 + 2	14 + 2	14 + 2

* Manufactured by Cutter Laboratories, Berkeley, Calif., USA.

[a] The full RIGH dose was given in a single intramuscular injection on day 0.

[b] DEV = rabies vaccine, duck embryo origin. Administered in 14 daily injections of 1 ml, followed by a 1-ml booster dose on day 23, and another on day 33.

As each subject presented himself for his first injection a pre-immunization blood sample was withdrawn, the subject was weighed, the dosage was determined from the chart, and the RIGH was administered intramuscularly. This inoculation was followed, if scheduled, by the first dose of DEV (1.0 ml given subcutaneously). The administration of DEV to those who were to receive it was uniform, consisting of 14 daily injections, each of 1.0 ml, beginning on day 0. An additional 1-ml booster injection was given on day 23 and another on day 33. These booster injections were in accordance with the regimen recommended by the WHO Expert Committee on Rabies (1966) to overcome interference by antirabies serum (equine origin).

In addition to the bleeding on day 0, serum samples were also obtained from all participants on days 1, 3, 6, 9, 23, 30, 40, 60, 90, and 180; a final sample will be taken 1 year after the first inoculation. The sera, which were frozen soon after their separation from the clot, were shipped frozen to the laboratory where they were kept frozen until tested. The laboratory received no information about the group to which each subject belonged. Each serum was identified simply by the subject's name and the date of bleeding.

Testing for serum-neutralizing antibody was begun when the 30-day sera from all participants became available. All samples from the same individual were tested at one time. The code was broken only after the 0–30-day specimens from all 41 participants had been tested. The 40-, 60-, and 90-day samples were then tested, the three sera from each subject being titrated simultaneously, repeating the 30-day sample in the same test.

Group A. As was indicated in Table 2, the 8 participants in group A received RIGH only, the dosage being 40 IU/kg. The results provided the rate of decline, or half-life, of the passively acquired homologous antibody. The antibody titres of the 0–90-day sera are shown in Table 3 for each individual, together with the ranges in titre obtained on each of the bleeding dates and the geometric mean titre calculated for those dates. The ranges in titre and the geometric mean titres are shown in Fig. 1.

All participants were seronegative for rabies antibody at the initiation of the study but 24 hours after RIGH injections all subjects had measurable antibody titres. The geometric mean titre was 1 : 11, with individual titres ranging from 1 : 8 to 1 : 16. The antibody level rose gradually to a peak geometric mean of 1 : 30 by the 13th day, when it began to decline. The period of antibody rise was longer than had been expected, and no obvious reason for this has been found. Nevertheless, the rate of decline of the antibody, from a peak titre of 1 : 30 at 13 days to a titre of 1 : 3 77 days later, conforms to the 21-day half-life estimated for homologous passive immune globulin in man.

Group B. The 8 subjects in this group received DEV only. As shown in Table 4 and Fig. 2, no rabies antibody was detected in any for 3 days after the initial vaccine injection. Low levels could be measured on the 6th day in only 3 of the participants and higher titres were obtained in 7 out of 8 on the 9th day.

All the subjects were positive for rabies antibody on the 13th day, their individual titres ranging from 1 : 9 to 1 : 128. Most titres continued to rise, reaching a peak geometric mean of 1 : 145 on day 40. Thereafter the mean decreased to one-half by day 90, although some individual titres remained quite elevated.

Group C. The subjects in group C were given 40 IU/kg of RIGH and a full course of DEV. The development of rabies antibody titres is shown in Table 5 and Fig. 3. As in groups A and B, all subjects in group C were seronegative before immunization. After 24 hours their geometric mean titre was 1 : 13, a level comparable with that of group A subjects after the same time interval. Also, as in group A, the geometric mean titres continued to rise

Table 3. Serum-neutralizing titres in group A subjects receiving rabies immune globulin (human) (RIGH) only

Subject	Serum-neutralizing titres[a] on the following days after the administration of RIGH : [b]										
	0	1	3	6	9	13	23	30	40	60	90
1. JB	< 2	9	18	16	50	32	20	17	20	7	3
2. SD	< 2	11	18	25	23	16	27	21	17	12	5
3. LH	< 2	8	13	36	14	36	25	11	9	9	< 2
4. TK	< 2	14	22	36	25	28	25	19	7	7	< 2
5. HM	< 2	11	16	35	45	35	28	26	20	11	4
6. MM	< 2	8	25	20	18	16	23	17	18	20	9
7. CM	< 2	16	22	25	22	50	25	25	25	8	3
8. NW	< 2	14	26	11	25	45	16	9	8	3	< 2
titre ranges	< 2	8–16	13–26	11–36	14–50	16–50	16–28	9–26	7–25	3–20	< 2–9
geometric means	< 2	11	20	24	26	30	23	17	14	9	3

[a] Reciprocal of dilutions.
[b] Dosage : 40 IU/kg.

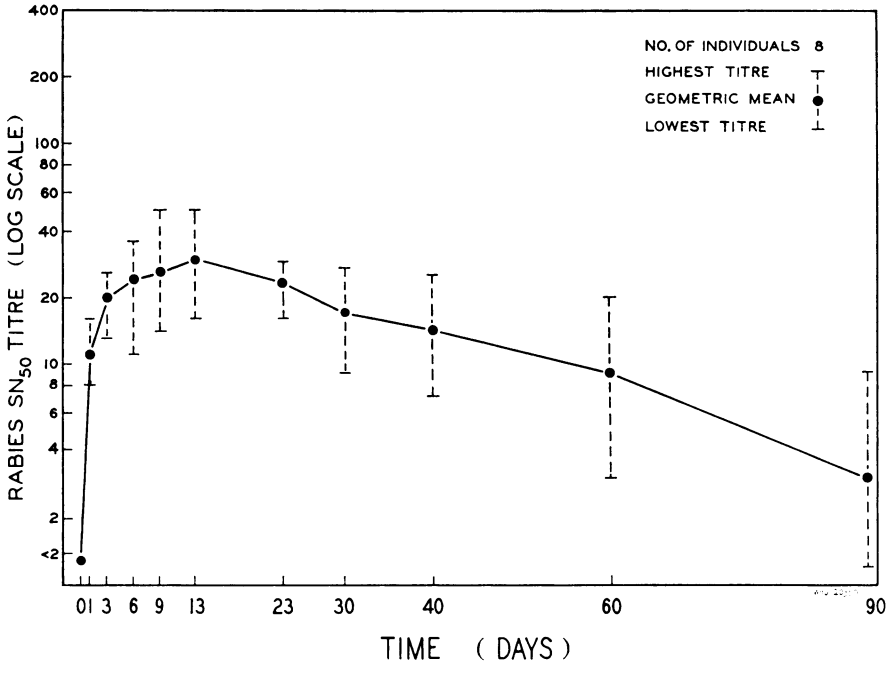

Fig. 1. Serum-neutralizing antibody response (SN_{50}) in group A subjects after the administration of rabies immune globulin (human) (RIGH) at a dosage of 40 IU/kg.

Table 4. Serum-neutralizing titres in group B subjects receiving rabies vaccine of duck-embryo origin (DEV) only

Subject	Serum-neutralizing titres[a] on the following days after the first injection of DEV:										
	0	1	3	6	9	13	23	30	40	60	90
1. MF	< 2	< 2	< 2	7	11	72	101	101	101	128	79
2. JH	< 2	< 2	< 2	4	10	40	44	36	51	36	29
3. KJ	< 2	< 2	< 2	2	14	79	320	454	513	363	255
4. RN	< 2	< 2	< 2	< 2	22	128	100	357	144	229	114
5. BP	< 2	< 2	< 2	< 2	26	100	199	360	507	403	227
6. JR	< 2	< 2	< 2	< 2	40	89	288	361	313	361	255
7. AS	< 2	< 2	< 2	< 2	< 2	9	14	18	51	14	13
8. SV	< 2	< 2	< 2	< 2	8	40	80	72	64	73	64
titre ranges	< 2	< 2	< 2	< 2–7	< 2–40	9–128	14–320	18–454	51–513	14–403	13–255
geometric means	< 2	< 2	< 2	2	11	56	99	133	145	124	87

[a] Reciprocal of dilutions.

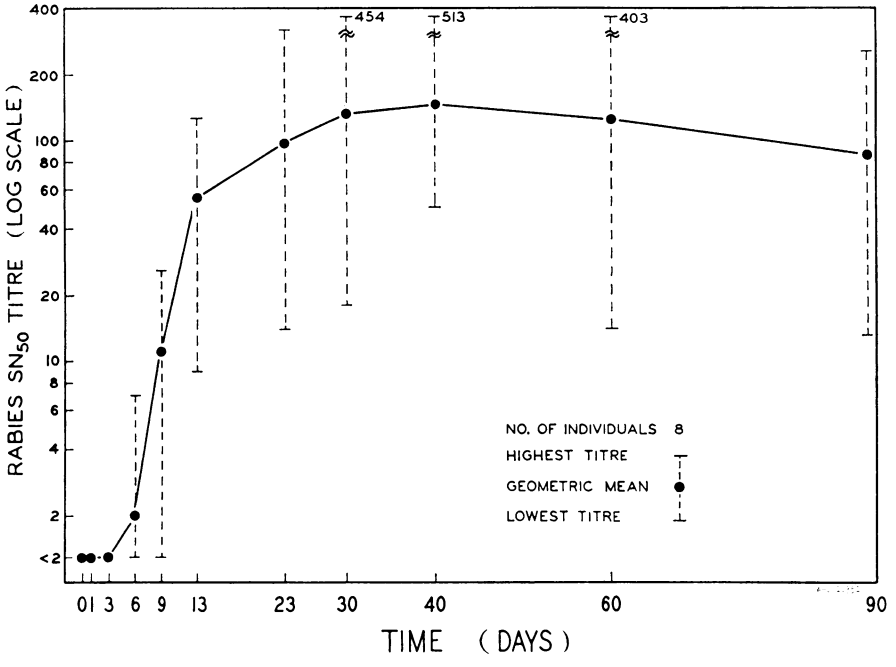

Fig. 2. Serum-neutralizing antibody response (SN_{50}) in group B subjects after the administration of a course of rabies vaccine of duck-embryo origin (DEV).

Table 5. Serum-neutralizing titres in group C subjects receiving rabies immune globulin (human) (RIGH) and rabies vaccine of duck-embryo origin (DEV); the dosage of RIGH was 40 IU/kg

Subject	Serum-neutralizing titres[a] on the following days after first injection:										
	0	1	3	6	9	13	23	30	40	60	90
1. RB	< 2	11	28	32	37	40	16	21	51	23	23
2. MB	< 2	20	40	40	20	28	32	36	36	28	10
3. RF	< 2	20	32	32	36	64	25	30	40	23	32
4. MR	< 2	3	25	22	32	32	23	54	91	45	45
5. TS	< 2	13	18	45	20	28	28	13	16	13	13
6. SS	< 2	20	20	32	70	126	203	157	114	72	—
7. ST	< 2	18	28	32	28	32	22	21	32	14	11
8. CT	< 2	16	40	40	323	643	641	570	513	641	647
titre ranges	< 2	3–20	18–40	22–45	20–323	28–643	16–641	13–570	16–513	13–641	10–647
geometric means	< 2	13	28	34	43	60	47	48	61	39	31

[a] Reciprocal of dilutions.

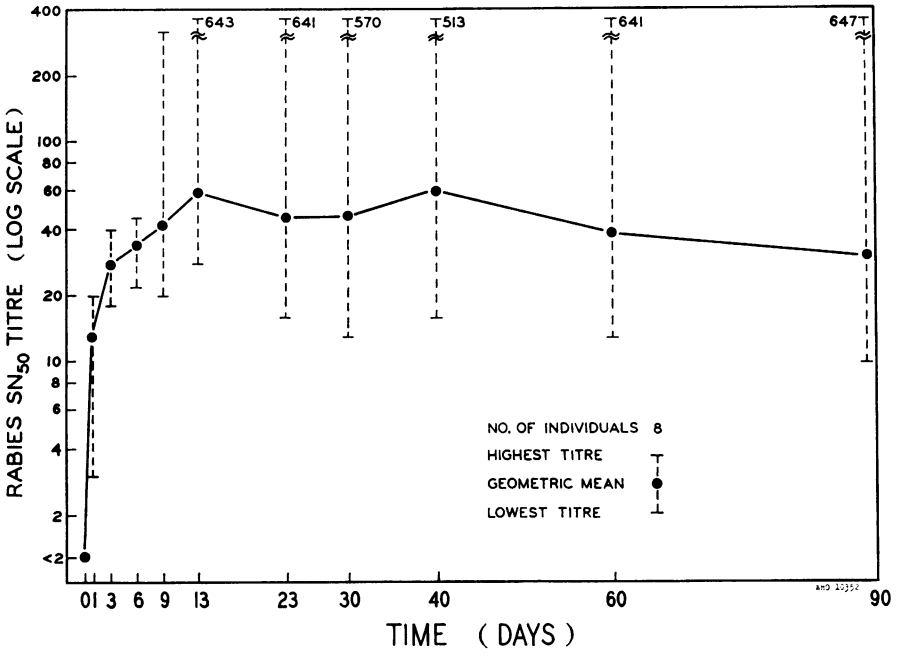

Fig. 3. Serum-neutralizing antibody response (SN_{50}) in group C subjects after the administration of rabies immune globulin (human) (RIGH) and a course of rabies vaccine of duck-embryo origin (DEV); the dosage of RIGH was 40 IU/kg.

until day 13. After a slight decline on days 23 and 30, the titres resumed their rise, reaching a peak on day 40, probably as a result of the booster injections on days 23 and 33. Titres were lower on days 60 and 90. Although the geometric mean titre on day 90 was about 10 times that of group A subjects on the same day, it was less than half that achieved in group B; this result was recorded in spite of the fact that one of subjects (CT) had an exceedingly high antibody response. Since 7 of the 8 participants in group C had consistently lower titres between days 23 and 90 by comparison with those of group B subjects, it seems that a RIGH dose of 40 IU/kg probably did interfere with the full expression of active immunity.

Group D. Subjects in group D each received 20 IU/kg of RIGH and a course of DEV. Of the 9 subjects in this group, 1 (EW) was found to have a low but definite rabies antibody titre before any immunizing injection. This individual had no history of rabies vaccination. The rapid climb of his antibody level on day 3 suggested an anamnestic response to the vaccine. The titres of this subject were, therefore, omitted from the calculation of the geometric mean titres given in Table 6 and used to plot Fig. 4.

In the other 8 participants, rabies antibody was absent on the day of the first injection but was detected in all subjects 24 hours later. Its geometric mean titre then was 1 : 6, about half the level noted after the injection of 40 IU/kg. The geometric mean titres rose gradually, and continued to rise through day 40 to reach a value of 1 : 111; i.e., almost twice that of group C on the same day, and only slightly lower than that achieved by group B. The same titre relationships continued up to and beyond day 90, suggesting little or no interference in group D.

Group E. It is evident from Table 7 and Fig. 5 that passive rabies antibody appeared late and rose slowly in this group, being barely detectable 24 hours after the injection of vaccine. On days 3, 6, and 9, the titres were still much lower than the corresponding values for groups C and D. Although rabies antibody titres continued to rise steadily up to and beyond day 40, the antibody profile of this group resembled that of group C, between days 30 and 90 rather than that of group D. The significance of this finding is

Table 6. Serum-neutralizing titres in group D subjects receiving rabies immune globulin (human) (RIGH) and rabies vaccine of duck-embryo origin (DEV); the dosage of RIGH was 20 IU/kg

Subject	Serum-neutralizing titres [a] on the following days after first injection:										
	0	1	3	6	9	13	23	30	40	60	90
1. TA	< 2	6	16	40	31	22	82	23	57	40	20
2. SB	< 2	6	16	20	18	16	18	12	13	9	5
3. DB	< 2	6	18	22	13	51	127	227	509	509	255
4. CG	< 2	5	8	14	25	9	11	227	80	90	90
5. WH	< 2	8	17	—	32	18	24	72	90	90	40
6. GH	< 2	9	11	10	18	28	100	162	456	361	516
7. LL	< 2	4	9	16	8	18	20	23	90	72	32
8. BS	< 2	6	22	20	30	71	76	229	203	161	93
9. EW [b]	3.2	9	40	28	22	25	40	73	112	70	70
Titre ranges	< 2	4–9	8–22	10–40	8–32	9–71	11–127	12–229	13–509	9–509	5–516
Geometric means	< 2	6	14	19	20	23	41	74	111	94	59

[a] Reciprocal of dilutions.
[b] Omitted from titre ranges and from calculation of geometric mean titres.

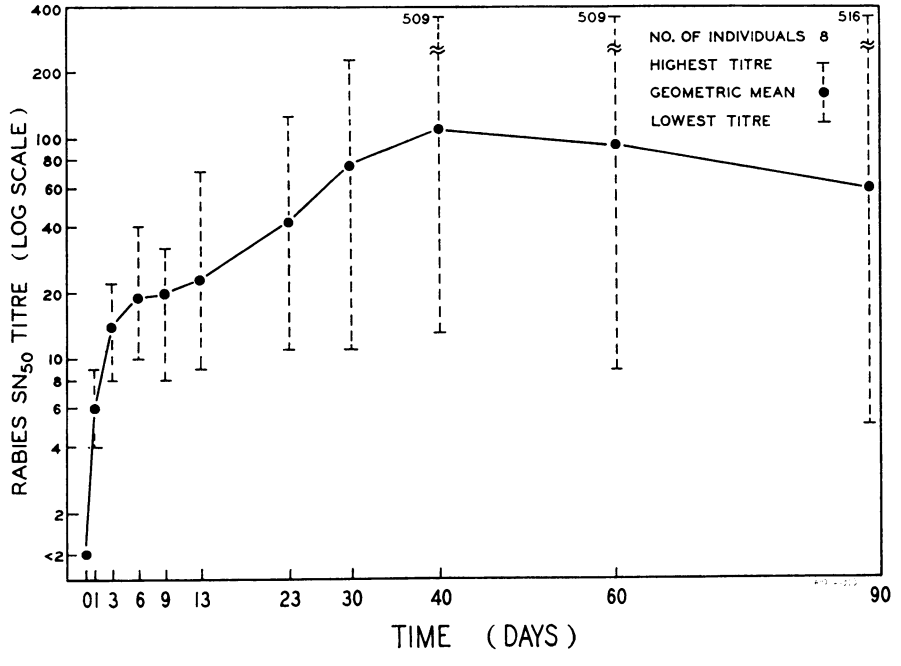

Fig. 4. Serum-neutralizing antibody response (SN_{50}) in group D subjects after the administration of rabies immune globulin (human) (RIGH) and a course of rabies vaccine of duck-embryo origin (DEV); the dosage of RIGH was 20 IU/kg.

Table 7. Serum-neutralizing titres in group E subjects receiving rabies immune globulin (human) (RIGH) and rabies vaccine of duck-embryo origin (DEV); the dosage of RIGH was 10 IU/kg

Subject	Serum-neutralizing titres[a] on the following days after first injection:										
	0	1	3	6	9	13	23	30	40	60	90
1. RA	< 2	4	13	14	26	40	36	25	80	101	45
2. KB	< 2	2	2	5	23	21	18	46	51	57	78
3. RH	< 2	< 2	3	4	7	13	28	30	57	64	40
4. MH	< 2	< 2	6	18	16	22	20	24	113	71	40
5. GR	< 2	< 2	4	4	15	20	22	65	143	201	57
6. RS	< 2	4	7	13	14	25	58	41	101	113	127
7. RT	< 2	3	7	14	20	32	20	16	25	20	32
8. RW	< 2	3	10	11	12	18	20	20	32	20	3
titre ranges	< 2	< 2–4	2–13	4–18	7–26	13–40	18–58	16–65	25–143	20–201	3–127
geometric means	< 2	2	6	9	16	23	26	30	65	63	37

[a] Reciprocal of dilutions.

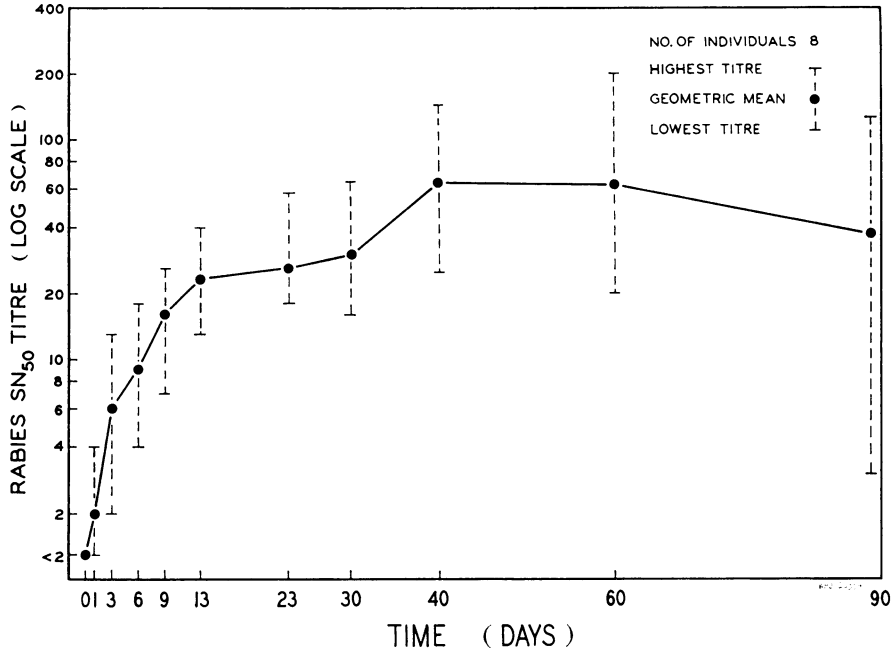

Fig. 5. Serum-neutralizing antibody response (SN_{50}) in group E subjects after the administration of rabies immune globulin (human) (RIGH) and a course of rabies vaccine of duck-embryo origin (DEV); the dosage of RIGH was 10 IU/kg.

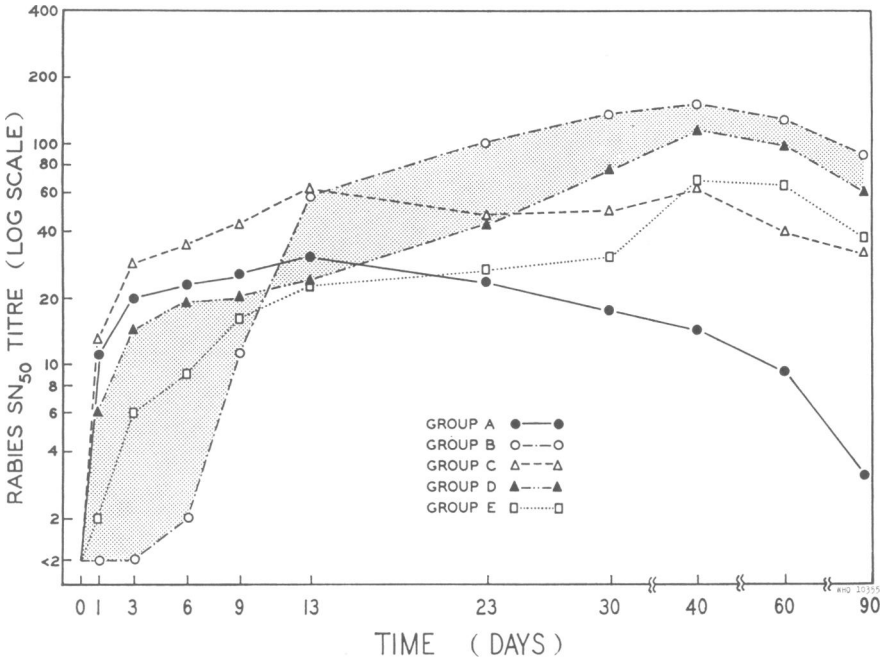

Fig. 6. Comparative neutralizing antibody responses (SN_{50}) in subjects of groups A–E following 5 different immunization schedules against rabies.

uncertain, however, because of the relatively small size of the study groups.

The trends of the comparative geometric mean titres of the five study groups shown in Fig. 6 again emphasize the apparent superiority of immunization schedule D. The seemingly lower active antibody titres achieved between days 30 and 90, in comparison with group B, are more than compensated for by the passive immunity induced between days 0 and 9 (Fig. 6, shaded area).

SUMMARY AND CONCLUSIONS

Donors with elevated rabies antibody titres were carefully selected from among human volunteer subjects who, because of previous exposure to rabid animals, had received one or more courses of rabies vaccine or who, because of the nature of their occupation, underwent actual pre-exposure immunization against rabies.

The results of antibody testing appear to justify the following conclusions:

(1) The half-life of RIGH in group A is in agreement with that estimated for homologous passive immune globulin in man.

(2) Twenty-four hours after the administration of 40 IU/kg and 20 IU/kg of RIGH, readily detectable levels of passive rabies antibody were found in all subjects. A dosage of 10 IU/kg, however, appears to be insufficient for early protection.

(3) Rabies antibody levels achieved by groups C and D between days 30 and 90 suggest that RIGH may have interfered with optimal active antibody production when the dosage was 40 IU/kg, while a dosage of 20/IU/kg results in minimal, if any, interference. Under the conditions of our study, 20 IU/kg may indeed represent an optimum dosage.

The active antibody response after a RIGH dose of 10 IU/kg, (group E) resembled that which followed 40 IU/kg (Group C), a rather unexpected result in view of the insufficient level of passive antibody.

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RÉSUMÉ

IMMUNOGLOBULINE ANTIRABIQUE D'ORIGINE HUMAINE: PRÉPARATION ET DÉTERMINATION DU DOSAGE CHEZ DES VOLONTAIRES NON EXPOSÉS À L'INFECTION

On a choisi des donneurs présentant des titres élevés d'anticorps antirabiques parmi des sujets qui par suite d'une exposition à l'infection avaient reçu une ou plusieurs séries d'injections de vaccin ou qui avaient bénéficié d'un traitement prophylactique en raison de leur profession.

Les plasmas prélevés chez ces donneurs ont servi à préparer deux lots d'immunoglobuline antirabique humaine (RIGH) possédant une activité supérieure à celle des préparations courantes d'origine équine. Le premier lot contenait 550 UI d'anticorps par millilitre et le second 240 UI/ml.

Afin de définir la dose optimale de RIGH à administrer en même temps que le vaccin antirabique préparé sur embryon de canard, 41 volontaires appelés à être prémunis contre la rage ont été répartis en 5 groupes et immunisés suivant l'un des schémas ci-après: *a*) 40 UI/kg de RIGH (groupe A); *b*) 14 injections de vaccin antirabique, suivies de 2 injections de rappel (groupe B); *c*) schéma *b* plus une dose de 40 UI/kg de RIGH (goupe C); *d*) schéma *b* plus une dose de 20 UI/kg de RIGH (groupe D); *e* schéma *b* plus une dose de 10 UI de RIGH (groupe E). Tous les participants ont fourni du sérum pour le titrage des anticorps antirabiques aux jours 0, 1, 3, 6, 9, 23, 30, 40, 60, 90 et 180.

Les résultats de ces titrages autorisent un certain nombre de conclusions. La demi-vie de la RIGH, chez les sujets du groupe A, est conforme aux prévisions et de l'ordre de 21 jours. Vingt-quatre heures après l'administration de 40 ou 20 UI/kg de RIGH, on décèle aisément la présence des anticorps antirabiques. Il n'en va pas de même avec une dose de 10 UI/kg qui se révèle incapable de protéger précocement contre l'infection. D'après les résultats des titrages effectués entre les 30e et 90e jours chez les sujets des groupes C et D, il semble qu'à la dose de 40 UI/kg la RIGH inhibe la réponse immunitaire active alors que l'interférence est très faible ou nulle si la dose est de 20 UI/kg. Dans les conditions de la présente étude, la dose de 20 UI/kg donne les meilleurs résultats.

La réponse immunitaire active, chez les sujets traités par une dose de 10 UI/kg de RIGH (groupe E), a été très semblable à celle suscitée par l'administration de 40 UI/kg (groupe C).

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