THE HALF-LIFE OF HOMOLOGOUS GAMMA GLOBULIN (ANTIBODY) IN SEVERAL SPECIES*

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The ideal method of determining the life of antibody molecules in the absence of immune reactions is to measure by quantitative immunochemical methods the rate of loss of passively administered homologous antibody. Such observations have been made in several species, as shown in Table I (1–7). However, the passive administration of enough homologous antibody for immunochemical studies is not always possible. Where it is not, the rate of utilization of I^{131}-labelled homologous gamma globulin can be used as a measure of the rate of utilization of antibody, as will be discussed.

Materials and Methods

Gamma globulins prepared by alcohol fractionation were generously supplied to us as follows: human from adult blood—Cutter Laboratories; human from cord blood—Lederle Laboratories; and bovine, rabbit, and dog—Armour and Company. Gamma globulins from mouse, rabbit, guinea pig, and monkey sera were isolated by ammonium sulfate fractionation of diluted sera at 0–3°C. as described by Sternberger and Petermann (8). Two additional fractionations at 35 per cent saturation with ammonium sulfate were introduced to insure removal of contaminating proteins.

The various gamma globulins were labelled with I^{131} in a carbonate buffer at pH 10, as previously described (9). Final preparations contained less than one iodine atom per molecule of protein. Approximately 99 per cent of the I^{131} was protein-bound, as determined by trichloracetic acid protein precipitation.

The following subjects were used:—

Eleven children varying in age from 6 months to 8 years.

Fourteen human adults between the ages of 32 and 72.

Two steers and two non-lactating cows weighing between 140 and 230 kg.

Eight adult mongrel dogs weighing between 8 and 20 kg.

Ten *Macacus rhesus* monkeys weighing between 1.8 and 4.9 kg.

Eighteen young albino male rabbits weighing between 2 and 2.2 kg. for gamma globulin half-life study.

Five young albino male rabbits weighing between 2 and 2.2 kg. to measure antibody disappearance rate.

Ten young albino male rabbits weighing between 2 and 2.2 kg. to study the relation of metabolic rate to globulin half-life.

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Thirteen hybrid guinea pigs weighing between 690 and 840 gm.

Twenty-seven young adult CFW strain female mice weighing from 21 to 22 gm.

These subjects were given oral potassium iodide supplements to saturate iodine utilizing tissues and minimize retention of I^{131} liberated by catabolism of labelled gamma globulin.

Procedure

In the determination of the half-lives of gamma globulin, I^{131}-labelled homologous gamma globulin was injected intravenously into all species except the mouse, in which the intraperitoneal route was used. No more than 5 mg. of protein per kg. body weight was used. Sufficient time was allowed for equilibration of the labelled globulin between intravascular and extravascular components of the plasma protein pool before the half-life determinations were started. This equilibration period appeared to vary directly with the size of the species.

TABLE I

Immunologic Determination of the Half-Life of Passively Administered Homologous Antibody

Animal	Investigator	Antibody	Half-life in days
Mouse	Hammon (1)	Japanese B encephalitis	1.9
Rabbit	Glenny and Hopkins (2)	Diphtheria antitoxin	4–5*
	Heidelberger *et al.* (3)	Type 1 antipneumococcus	4.2*
	Germuth *et al.* (4)	Anti egg albumin	4.7*
Horse	Glenny and Hopkins (2)	Diphtheria antitoxin	19–25*
Human newborn	Neill *et al.* (5)	Diphtheria antitoxin	35
		Antibacterial (diphtheria)	30
	Barr *et al.* (6)	Diphtheria antitoxin	31
	Wiener (7)	Rh antibody	30

* Half-life values are our calculations based on data in original paper.

After equilibration all subjects except mice were bled 1 cc. to 2 cc. at regular intervals, and the concentration of protein bound I^{131} in the plasma was determined by Geiger count for each individual (9). These determinations were made at weekly intervals in beef and human beings and at biweekly intervals in dogs, monkeys, rabbits, and guinea pigs. Since the rate of labelled globulin utilization did not vary significantly during the periods of observation, the half-life for each individual for the total time of observation was used in calculating the averages and standard deviations presented in Table II. The 27 mice received identical injections of labelled protein and nine were exsanguinated the 1st day after injection, nine the 3rd day, and nine the 5th day. The blood from the mice in each group was pooled, and the labelled protein concentration in the pooled plasma was determined. The half-life value for mice in Table II was calculated from these three pooled plasma determinations.

The half-life of the decline of serum antibody during a period following active immunization when relatively little antibody production occurs should approach the true half-life of the antibody molecule. To obtain such a value for comparison with the I^{131} homologous gamma globulin half-life, we measured the decline of serum antibody concentration in five rabbits between the 10th and the 17th day after intravenous injection of 60 mg. of bovine gamma

TABLE II

Half-Life of I^{131} Labelled Homologous Gamma Globulin

Species	No. of subjects	Method of fractionation	Interval during which half-life is calculated after injection	Average half-life of gamma globulin with standard deviation
			days	*days*
Beef	4	Alcohol	6–27	21.2 ± 1.7
Human				
6 mo.–8 yrs.	11	Alcohol	4–30	20.3 ± 4.1
Adult	14	"	4–21	13.1 ± 2.8
Dog	8	Alcohol	7–17	8.0 ± 1.1
Monkey	10	Ammonium sulfate	4–24	6.6 ± 1.6
Rabbit	9	Alcohol	4–21	4.6 ± 0.8
	9	Ammonium sulfate	4–21	5.7 ± 1.2
Guinea pig	13	Ammonium sulfate	4–13	5.4 ± 0.9
Mouse	27	Ammonium sulfate	1–5	1.9

TABLE III

Rate of Disappearance of Antibovine Gamma Globulin Following Maximum Antibody Concentration in Actively Immunized Rabbits

Animal No.	Anti-BGG Concentration			Antibody disappearance half-lives		
	Length of time after immunizing injection					
	Days..... 10	14	17	10–14	14–17	10–17
	μg. N/ml. serum	*μg. N/ml. serum*	*μg. N/ml. serum*	*days*	*days*	*days*
211	132.0	86.0	61.7	6.5	6.2	6.4
212	91.1	62.8	30.6	7.4	2.9	4.4
213	142.0	81.0	51.1	4.9	4.5	4.7
214	108.7	63.1	37.4	5.1	4.0	4.6
215	162.6	86.6	64.8	4.4	7.2	5.3
Average.....				5.6 ± 1.1	5.0 ± 1.6	5.1 ± 0.7

globulin (Table III). Antibody determinations were made by the quantitative immunochemical methods described by Heidelberger and Kendall (10).

The findings of the above studies led us to the hypothesis that the life of gamma globulin is in part a function of the metabolic rate of the host; and to test this hypothesis, we determined the gamma globulin half-life in ten thyroxin-treated rabbits. Thyroxin was injected in

doses of 0.2 mg. per kg. body weight per day over the 12 day period of observation. This regimen maintained the oxygen consumption per kilogram body weight at approximately twice the normal rate as measured by a Benedict-Roth metablor connected to a sealed animal cage.

RESULTS AND DISCUSSION

The average half-lives of homologous gamma globulin labelled with I^{131} were as follows in the seven species studied: beef, 21.2 days; human children (6 months to 8 years), 20.3 days; human adults, 13.1 days; dogs, 8.0 days; monkeys, 6.6 days; rabbits, 4.6 and 5.7 days; guinea pigs, 5.4 days; and mice, 1.9 days (Table II).

TABLE IV

Half-Life of Rabbit Antibody and Gamma Globulin

Passively administered homologous antibody (immunologic determination)

		days
Glenny and Hopkins (2)	Diphtheria antitoxin	4–5
Heidelberger *et al.* (3)	Type 1 antipneumococcus	4.2
Germuth *et al.* (4)	Antiegg albumin	4.7

I^{131} labelled homologous gamma globulin (radioactivity determination)

	days
Alcohol fractionated gamma globulin	4.6
Ammonium sulfate fractionated gamma globulin	5.7

Autologous antibody—disappearance following peak concentration during active immunization (immunologic determination)

		days
Taliaferro and Taliaferro (11)	Hemolysin	5.5
	Antibovine gamma globulin	5.1

The rabbit and mouse offer a basis for comparison between the half-lives of gamma globulin determined by isotope techniques and the half-lives of antibody determined by immunological techniques. In the rabbit the gamma globulin half-lives (4.6 and 5.7 days) agree well with the passively transferred antibody half-lives (4 to 5 days) (2–4) (Table IV). Taliaferro and Taliaferro (11) used a third method of approximating antibody half-life in the rabbit: measurement of decline of serum antibody following active immunization as described under our Procedure. They obtained a half-life of 5.5 days for the hemolysin decline after immunization with heterologous erythrocytes (Table IV). The authors found a 5.1 day half-life for the decline of antibovine gamma globulin in the period immediately following the peak antibody concentration (Tables III and IV). Our half-life of homologous gamma globulin in the mouse, 1.9 days, agrees exactly with the half-life of passively administered homologous

antibody to Japanese B encephalitis in the mouse as determined by Hammon (1). It would appear from the agreement between half-lives of homologous gamma globulin and antibody in the rabbit and mouse that these half-lives in other species might be similar. If so, in species where the immunochemical determination of the life of antibody is not feasible, I^{131}-labelled gamma globulin life could be used as an approximation of antibody life.

From Table II it is apparent that the half-life of gamma globulin varies directly with the size of the species and—of considerably more significance—inversely with the metabolic rate expressed per unit weight (12). It is logical that the rate of catabolism of gamma globulin should be related to the metabolic rate of the host. To test this assumption, homologous gamma globulin labelled with I^{131} was traced in ten thyroxin-treated rabbits with metabolic rates approximately twice normal, and the half-life was found to be 3.2 ± 0.4 days—significantly lower than the control value of 4.6 ± 0.8 days. Since the gamma globulin half-lives in different species vary inversely with the metabolic rate of the species, and since elevation of metabolic rate shortens the gamma globulin half-life of a given species, it would appear that gamma globulin half-life (and therefore antibody half-life) is in part dependent on the metabolic rate.

The metabolic rate, however, is not the only factor determining the globulin or antibody half-life, as is evident from our observations in human beings. The globulin half-life in children (20 days) is longer than in adults (13 days) in spite of the fact that the metabolic rate in children is higher. Factors other than metabolic rate and perhaps associated with age must be responsible for this difference.

The dependence of globulin half-life on both unknown factors and metabolic rate makes it difficult to compare our 20 day globulin half-life in children with the 30 day antibody half-life found in newborns (5–7). Immediately after birth the metabolic rate is low. Several months later it increases to its maximum, which is approximately double the newborn rate, following which it decreases slowly throughout life (12). It may be that the long antibody half-lives found in the first few months of life are the result of this low initial metabolic rate.

SUMMARY

1. The half-lives of homologous gamma globulins labelled with I^{131} agree well with the half-lives of passively administered homologous antibodies and provide a simple, quantitative tool for the determination of antibody half-life when immunochemical techniques are difficult to apply.

2. The half-lives of gamma globulin or antibody vary markedly from species to species and apparently even among age groups within a species.

3. The half-lives of gamma globulin or antibody seem to be dependent, at least in part, on the metabolic rate of the host.

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