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Estimation of the intravascular half-lives of normal rhesus monkey IgG, IgA and IgM

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Summary. Rhesus monkey IgG, IgA and IgM were purified from pooled normal serum and labelled with ^{125}I. Four monkeys were injected intravenously with approximately 50 μg of each purified immunoglobulin containing between 25 μCi and 50 μCi of ^{125}I. Iodination was approximately 1 atom per 14 molecules of IgG and IgA and per 2·5 molecules of IgM. Samples of serum were taken for up to 22 days after injection and radioactivity compared with that in a sample taken within 30 min of injection. Sucrose density ultracentrifugation of serum samples revealed that in animals given IgG or IgA, radioactivity was associated with 7S protein peaks, and in animals given IgM radioactivity was associated predominantly with a 19S protein peak. Most of the radioactivity could be precipitated from serum samples with the appropriate monospecific antiserum. The mean half-life of rhesus monkey IgG was estimated as 8·3 days, IgA as 4·5 days and IgM as 4·7 days.

INTRODUCTION

The rhesus monkey provides a valuable model for many human diseases since its anatomy and physiology is essentially similar to that of man. It is therefore possible to relate findings to the human situation with greater confidence than with other animal models. The rhesus monkey has been widely used in the study of organ and tissue transplantation, and the major histocompatibility complex in particular has been thoroughly investigated (Balner, Gabb, Dersfant, van Vreeswijk & van Rood, 1971; Rogentine, Vaal, Ellis & Darrow, 1971). The rhesus monkey is also used in the study of immunity against infective diseases including malaria (Schenkel, Simpson & Silverman, 1973; Cohen, Butcher, Mitchell, Deans & Langhorne, 1977) and dental caries (Lehner, Challacombe & Caldwell, 1975, 1976).

Immunoglobulin concentrations in the serum of rhesus monkeys are in the same range as those in man (Monte-Wicher, Wicher & Arbesman, 1970; Russell & Bergmeier, unpublished) when assayed using antisera raised against purified monkey IgG, IgA and IgM. The immunoglobulin concentration in parotid and mixed saliva appears to be comparable to that found in man (Challacombe, Russell & Hawkes, 1978). In contrast little is known of the metabolism of immunoglobulins in the rhesus monkey. Dixon, Talmage, Maurer & Deichmiller (1952) estimated the half-life of rhesus gammaglobulin to be between 6 and 7 days, but no separate estimations of the half-lives of IgG, IgA or IgM have been carried out. As part of a study of the effects of passive transfer of antibodies by different

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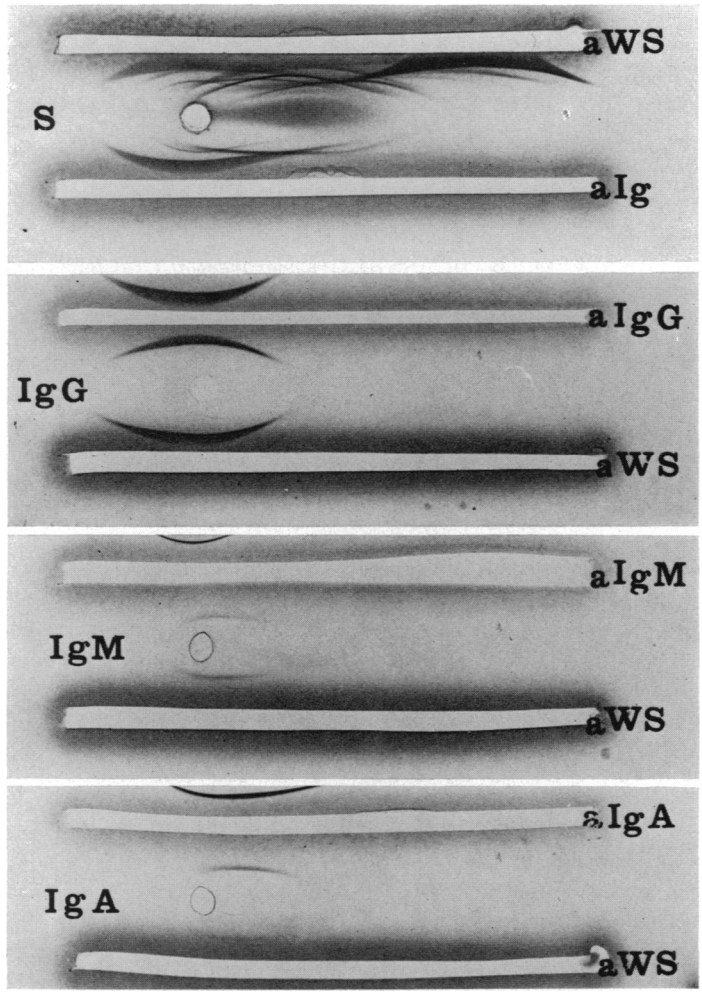


Figure 1. Immunoelectrophoresis of purified rhesus monkey IgG, IgA and IgM: wells, purified immunoglobulin preparations; troughs, rabbit anti-rhesus monkey serum or rabbit antimonkey IgG, IgM or IgA.

immunoglobulin isotypes (Lehner, Russell, Challacombe, Wilton, Scully & Hawkes, 1978), the intravascular half-lives of purified rhesus monkey IgG, IgA and IgM were examined.

MATERIALS AND METHODS

Monkey immunoglobulins

Rhesus monkey immunoglobulins were isolated from pooled normal monkey serum. IgG was purified by a combination of DEAE cellulose chromatography and gel filtration, IgM by gel filteration and zone electrophoresis and IgA by gel filtration, DEAE cellulose chromatography, hydrophobic chromatography and zone electrophoresis as described in detail elsewhere (Russell & Bergmeier, unpublished). Each immunoglobulin gave a single precipitation line against a rabbit antiserum raised against normal monkey serum (Fig. 1). The purified immunoglobulins were labelled with ^{125}I (IMS-3, Radiochemical Centre, Amersham) by a modification of the chloramine T method as described elsewhere (Challacombe *et al.*, 1978a). Under these

conditions a maximum substitution of 1 atom of iodine per 14 molecules of IgG and IgA, and 1 atom of iodine per 2·5 molecules of IgM was achieved.

Injection of labelled immunoglobulins

Twelve rhesus monkeys, which weighed between 3·0 and 4·7 kg, were injected with radiolabelled immunoglobulins into the small (short) saphenous vein of the hind limb; groups of four monkeys were given IgG, IgA or IgM. The animals were given approximately 50 μg of the purified immunoglobulin containing between 25 μCi and 50 μCi of ^{125}I. A sample of blood was taken within 30 min of injection and thereafter at intervals for up to 22 days.

Sucrose density gradient rate-zonal ultracentrifugation

Sucrose density gradients were prepared as described previously (Challacombe, Russell, Hawkes, Bergmeier & Lehner, 1978b). For ultracentrifugation of IgG samples, linear 10–25% w/w gradients were used, for IgA 10–30%, and for IgM 10–40%. Samples of 20 μl of radioactive serum, taken 1 h or 24 h after intravenous injections of the labelled immunoglobulin preparations, were made up to 100 μl with saline and centrifuged at 65,000 rev/min for 16 h at 4°. Fractions were collected and assayed for radioactivity and protein concentration as previously described (Challacombe *et al.*, 1978a). The positions and concentrations of IgG, IgA and IgM were determined by single radial immunodiffusion.

Immune precipitation of radioactivity in serum samples

Samples of serum (10 μl) taken 1 h after intravenous injection of the radiolabelled immunoglobulins were mixed with 50, 100 and 150 μl of the appropriate antisera to monkey immunoglobulin (Russell & Bergmeier, unpublished) and the volume made up to 200 μl with saline. After incubation for 1 h at 37° and 18 h at 4° the samples were centrifuged for 10 min at 2000 *g*. The precipitate was washed three times in saline and the radioactivity in the pellet and the supernatant (including the three washes) was counted.

RESULTS

Ultracentrifugation

After injection of radiolabelled IgG, approximately 99% of the ^{125}I count of serum was located in the 7S zone on sucrose density gradient anlaysis (Fig. 2a). All the IgG detected by single radial diffusion was also present in this zone. In animals given labelled IgA, the 7S zone was found to contain all of the serum radioactivity and all of the serum IgA (Fig. 2b). Ultracentrifugation of samples from animals given labelled IgM on a 10 to 40% sucrose density gradient resulted in a protein peak of approximately

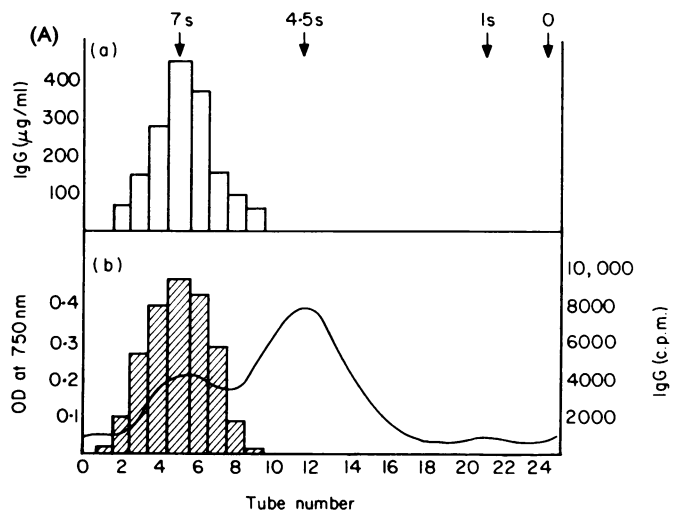
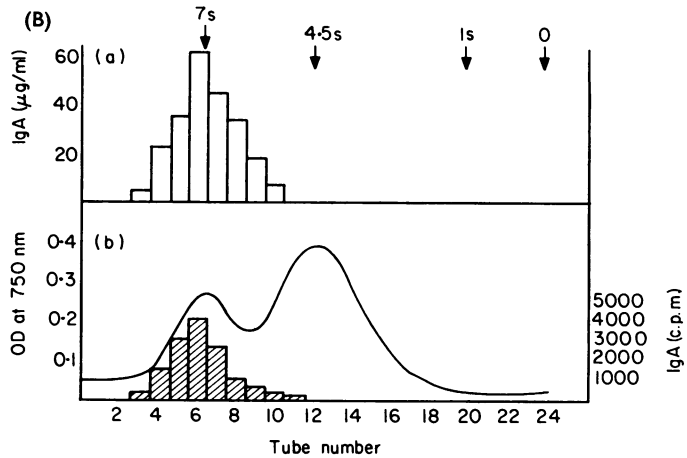
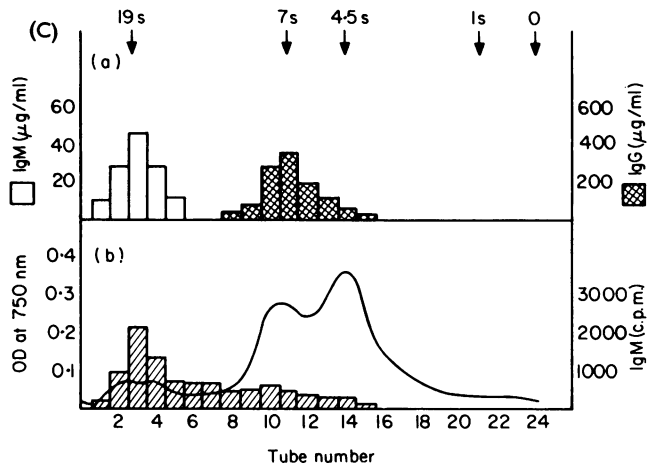


Figure 2. Sucrose density ultracentrifugal analysis of serum from rhesus monkeys taken 1 hour after intravenous injection of radiolabelled immunoglobulins. (A) Concentration of IgG (a) and radioactivity (b) in fractions of serum from animals given radiolabelled IgG (10–25% gradient).



(B) Concentration of IgA (a) and radioactivity (b) in fractions of serum from animals given radiolabelled IgA (10–30% gradient)



(C) Concentration of IgG and IgM (a) and radioactivity (b) in fractions of serum from animals given radiolabelled IgM (10–40% gradient).

19S in addition to the 7S and 4·5S peaks. Serum IgM and most of the radioactivity was found in the 19S zone, but in addition some activity was present in the 7S zone (Fig. 2c).

Immune precipitation

The maximum amount of radioactivity was precipitated with the greatest amount (150 μl) of antiserum with each immunoglobulin. In animals injected with labelled IgG, 98% of the serum radioactivity was precipitable with anti-IgG serum. Anti-monkey IgA precipitated 87% of the serum activity from animals given labelled IgA and anti-monkey IgM serum precipitated 67% of the serum activity in animals given labelled IgM.

Intravascular half-lives and extrapolations

The serum levels of radioactivity were expressed as a percentage of the counts in the samples taken within 30 min of injection of the radiolabelled IgG, IgA or IgM, and are shown (Fig. 3). Correction was made for the decay rate of ^{125}I. With each immunoglobulin preparation a sharp fall in serum radioactivity was found over the first 5 days with an exponential decline from approximately 7 days. After 22 days the mean level of radioactivity in the IgG

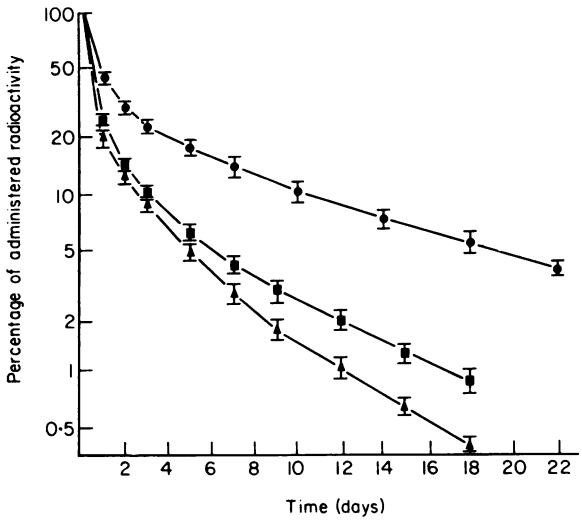


Figure 3. Semi-logarithmic plot of the time course of decline of serum radioactivity in animals given IgG (●), IgA (▲) or IgM (■). Mean ± SE of four animals in each group.

animals was 3·8% of the administered radioactivity. With animals given IgA serum radioactivity had fallen to 1% of the initial value by 12 days, and with animals given IgM was 1% of the initial value by 17 days (Fig. 3). Using the portion of the curve showing exponential decline of radioactivity, and obeying the formula $C = C_o e^{-kt}$ where C = the amount present at time t, C_o = the amount present initially, t = time and k is a constant, the half-life of IgG in the four animals ranged from 7·5 days to 8·7 days with a mean of 8·3 days. The half-life of IgA ranged from 4·2 days to 5·2 days with a mean of 4·5 days and the half-life of IgM ranged from 4·4 days to 4·9 days with a mean of 4·7 days (Table 1).

Using the half-life estimations obtained, the fractional catabolic rate (Fcr) for each immunoglobulin was calculated using the formula $Fcr = (0{\cdot}693)/(T\frac{1}{2})$ (Sterling, 1951). Thus the mean Fcr for IgG was 8·4%, for IgA 15·5% and for IgM 14·8% of the total body IgG, IgA or IgM (Table 1).

The mean ± SD of the blood volume of the twelve animals was 86 ± 10 ml/kg and the mean plasma volume was 50 ± 6 ml/kg estimated by the dilution of the radioactivity administered by blood and plasma respectively. The vascular pools of each immunoglobulin were the product of the individual plasma pool (ml/kg) and the plasma concentration (mg/ml) to give mean values of 484 mg/kg for IgG, 160 mg/kg for IgA and 24 mg/kg for IgM (Table 1).

DISCUSSION

The intravascular half-life of normal rhesus monkey IgG was estimated to be 8·3 days. This compares

Table 1. Some characteristics of Rhesus monkey immunoglobulins

	Serum concentration mg/ml*	Vascular pool mg/kg†	Half-life days†	% of body content catabolized per day†
IgG	9·36 ± 0·53	484 ± 44	8·3 ± 0·54	8·4 ± 0·6
IgA	3·11 ± 0·42	160 ± 19	4·5 ± 0·46	15·5 ± 1·5
IgM	0·49 ± 0·11	24 ± 3	4·7 ± 0·26	14·8 ± 0·8

* Mean ± SD of twelve monkeys; estimated by single radial diffusion using rabbit anti-monkey immunoglobulin antisera.

† Mean ± SD of four monkeys.

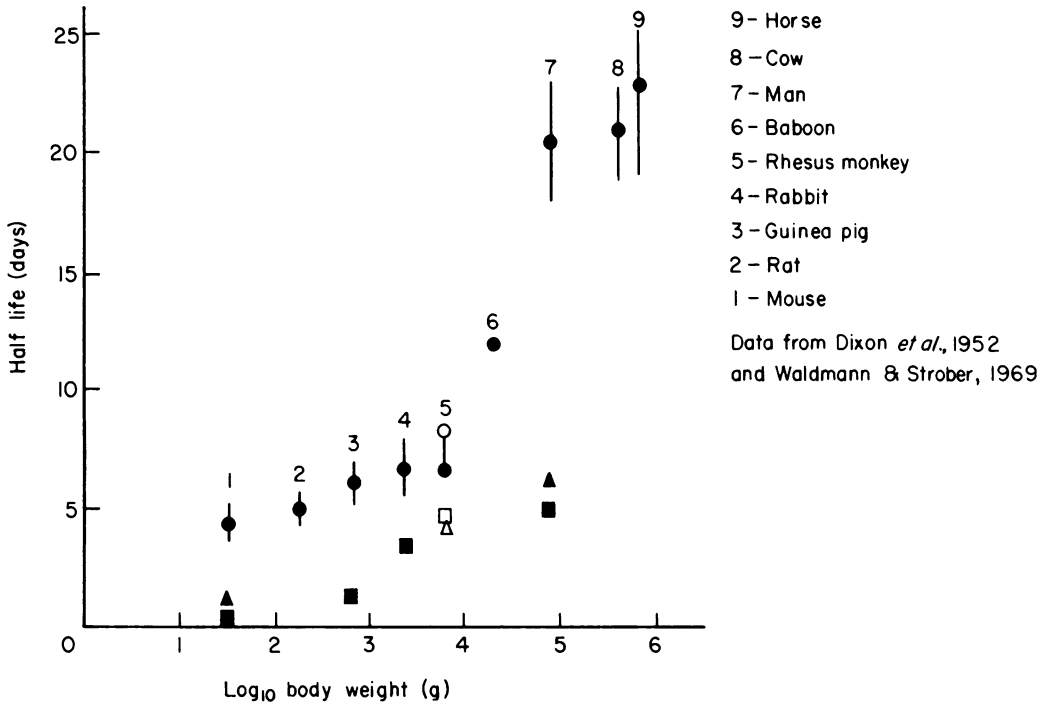


Figure 4. Half-lives of immunoglobulins in various species in relation to body weights in grams. Mean value and range of values for IgG (●) reported are shown, also values for IgA (▲) and IgM (■). Results of this study IgG (○), IgA (△) and IgM (□).

reasonably well with the 6·6 days reported for rhesus monkey gammaglobulin isolated by ammonium sulphate precipitation (Dixon *et al.*, 1952). This type of preparation would have probably contained IgA and IgM in addition to IgG and other serum proteins and would shorten the recorded half-life accordingly. The half-life of IgG increases with the average weight of the species from approximately 4 days in the mouse (Fahey & Sell, 1965) and 6 days in the rabbit (Cohen, Holloway, Mathews & McFarlane, 1956) to over 21 days in the cow (Dixon *et al.*, 1952) and 23 days in the horse (Glenny & Hopkins, 1922). The relationship between half-lives of IgG and body weight is shown in Fig. 4 and the estimation of 8·3 days for the half-life of IgG in the rhesus monkey agrees well with this general relationship.

For IgA the intravascular half-life was estimated as 4·5 days. This compares with 1 day in the mouse (Fahey & Sell, 1965) and 6 days in man (Strober, Wochner, Barlow, McFarlin & Waldmann, 1968). With IgM the estimated intravascular half-life of 4·7 days compared with 0·4 days in the mouse (Fahey & Sell, 1965) and 10 days in man (Rothschild & Waldmann,1970). There appear to be no other reports of the half-lives of IgA or IgM in rhesus monkeys.

Thus the half-lives of IgG, IgA and IgM reported in this paper closely approximate the values expected when comparing the rhesus monkey with other species (Fig. 4). Accurate determination of half-lives is dependent on many factors discussed fully by Waldmann & Strober (1969). The purity of the protein used is an essential prerequisite. All three immunoglobulins were pure as judged by immunoelectrophoresis (Fig. 1). However, this is a relatively insensitive method and does not exclude low levels of contaminating proteins. Sucrose density ultracentrifugation and immunoprecipitation were performed on serum samples taken after administration of labelled immunoglobulins to confirm the purity of the preparations and to ensure that gross damage had not occurred during the isolation procedure. With IgG and IgA all of the ^{125}I activity was found in the appropriate 7S immunoglobulin region (Fig. 2). With IgM, the majority of the activity was

in the 19S region, but in addition some activity was found in the 7S region. This may have been due to some de-polymerization of the IgM into 7S IgM sub-units following the radiolabelling procedure, or alternatively that some impurities such as α_2 macroglobulin sub-units were present in the IgM preparation. If impurities were present the half-life of IgM may be slightly overestimated. The IgM preparation did not react with anti-IgG, or anti-IgA antisera.

The possibility should be considered that purification and radiolabelling of the monkey immunoglobulin preparations may have altered their biological activity. The technique for protein isolation involving ion exchange chromatography, electrophoresis and gel filtration have generally proved satisfactory for metabolic studies (McFarlane, 1957, 1965; Freeman, 1959; Cohen, Freeman & McFarlane, 1961; Anderson, 1964). The use of a strong oxidizing agent such as Chloramine T may sometimes lead to altered biological activity (Waldmann & Strober, 1969) and iodination should be minimal with less than 1 atom of iodine per molecule of protein. Iodination of more than 2·5 atoms per molecules of protein may result in damage to the protein molecule with accelerated catabolism (Bloom, Crockett & Stewart, 1958; McFarlane, 1963). In this study iodination was minimal with a maximum value of 1 atom of iodine per 14 molecules of IgG or IgA and a maximum of 1 atom of iodine per 2·5 molecules of IgM. Self-radiation by the iodine isotope may be a major cause of protein denaturation (Yalow & Berson, 1957), and it is important to use the preparations without delay. In this study preparations were administered on the day of iodination. The possibility of altered biological activity cannot be excluded, although immune precipitation of serum samples after injection of the radiolabelled preparations showed that they were immunologically reactive since 98% of the activity in IgG samples and 87% of the activity in IgA samples could be precipitated. Only 67% of the activity in IgM samples could be precipitated and this may have been partly due to the presence of other contaminating proteins discussed above.

The calculated catabolic rate for monkey IgG is approximately three times higher than that in man (Rothschild & Waldmann, 1970), but is consistent with the finding that the half-life is only one-third of that in man given a similar serum concentration (Table 1). The half-lives, catabolic rate and serum concentrations of IgA and IgM appear to be similar in both monkey and man (Table 1).

The estimation of the intravascular half-lives of rhesus monkey immunoglobulins reported in this study, together with details on the catabolic rates and plasma volumes, should prove useful in determining the effects of passive transfer of antibodies of different immunoglobulin isotypes, and in other studies involving immunoglobulins or specific antibodies in monkeys.

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