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Serum disappearance and catabolism of homologous immunoglobulin fragments in rats

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SUMMARY

The serum disappearance, metabolic clearance and whole body catabolism of homologous immunoglobulin fragments were studied in rats. The rapid disappearance of Fab fragments from serum seen in normal animals was no longer present after nephrectomy. In contrast, the serum disappearance curve of Fc fragments was not altered by nephrectomy. The results of three different experiments, however, indicated that similar to Fab fragments, some Fc fragments underwent filtration and degradation in the kidneys. First, the amount of intact Fc fragments excreted in the first day after injection increased from 6% of the injected dose in normal rats to 17% in rats pretreated with sodium maleate. Secondly, nephrectomy eliminated the rapid phase of whole body catabolism of injected Fc fragments. Thirdly, autoradiographic studies showed localization of Fc fragments in the renal proximal tubule cells in the first 3 hr after injection. An identical localization was seen with Fab fragments.

These results support the conclusion that removal from circulation by glomerular filtration and subsequent reabsorption and degradation in proximal tubule cells represent the major mechanism for catabolism of Fab fragments. Although some Fc fragments undergo the same fate, most injected Fc fragments equilibrate with unknown sites where they are possibly bound and made unavailable for filtration in the kidneys.

INTRODUCTION

Although the Fc and Fab papain fragments of IgG are very similar in size, their serum disappearance characteristics have been shown to be markedly different. Previous studies have demonstrated that the disappearance of Fab from serum is very rapid, with a half-life of less than 1 hr (Spiegelberg & Weigle, 1965). In contrast, the serum disappearance curve of Fc possessed a slow component whose half-life was similar to that of whole IgG (Spiegelberg & Fishkin, 1972). These investigators assumed that the slowest phase of serum disappearance of an injected protein represented catabolism, after equilibration had occurred. Therefore, these results suggested that Fab was catabolized rapidly but that Fc was catabolized more slowly, at the same rate as IgG. Furthermore, the catabolism of the whole IgG molecule was dependent upon structures within the Fc portion of the molecule. Fc fragments, but not Fab, led to an increased catabolic rate for IgG when administered concurrently (Fahey & Robinson, 1963). These results indicated that intact IgG and Fc fragments may be catabolized by the same, or similar, mechanisms.

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The sites of catabolism for the intact IgG molecule, or for the various IgG fragments, are not known (Waldmann & Strober, 1969; Strober, Mogielnicki & Waldmann, 1973; Waldmann & Jones, 1973). Wochner, Strober & Waldmann (1967) showed that the kidneys were a major site of catabolism of light chain dimers, and may in part be responsible for Fab catabolism. These authors also concluded that rabbit Fc was not subjected to renal catabolism in mice.

The results of previous studies, however, failed to establish why Fc was not removed rapidly from serum by glomerular filtration, as were proteins of a similar size such as Fab or light chain dimers (Wochner *et al.*, 1967). The possible presence of aggregated proteins and the performance of IgG metabolic studies in heterologous systems may have influenced the results of past studies. The present experiments were undertaken in an effort to clarify the mechanisms of handling of Fc and Fab fragments by the body. Homologous Fc and Fab free of aggregates were infused into rats and their fate was determined by analysis of serum disappearance curves, metabolic clearance studies, whole body catabolic experiments, and autoradiography of tissue sections.

MATERIALS AND METHODS

Preparation of immunoglobulin fragments. Normal rat gamma-globulins (Cohn Fraction II, prepared from sera of Sprague–Dawley or Wistar strain rats) were purchased from Miles Laboratories, Kankakee, Illinois, and from Schwarz–Mann, Orangeburg, New York. Monomeric IgG was obtained from these preparations by gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Incorporated, Piscataway, New Jersey) equilibrated with borate buffer (0·2 M sodium borate, 0·15 M NaCl, pH 8·0). This preparation contained only IgG as demonstrated by double diffusion studies in agar using rabbit anti-rat serum (Schwarz-Mann) and goat anti-rat IgG (Miles Laboratories). Rat light (L) chains were obtained by reduction and alkylation of purified IgG and subsequent gel filtration on a Sephadex G-200 column equilibrated with 0·5 M propionic acid.

Papain digestion of rat IgG was carried out using modifications of the method described by Porter (1959). Monomeric rat IgG (20 mg/ml in 0·01 M acetate, pH 5·5) was preincubated with 0·01 M cysteine and 0·002 M EDTA for 2 hr at 37°C. Mercuripapain (Worthington Biochemical Corporation, Freehold, New Jersey) was added to give an enzyme:protein ratio of 1:100 by weight. After incubation for 15 min at 37°C, the papain digestion was stopped by making the mixture 0·011 M in *N*-ethyl maleimide or 0·011 M in iodoacetamide. The solution was passed over a Sephadex G25 column at 4°C, equilibrated with 0·01 M phosphate buffer, pH 7·8. The papain digest was then applied to a diethylaminoethyl cellulose column (DE 52, Whatman), equilibrated with the same buffer. The DE 52 effluent contained pure Fab as determined by double diffusion studies in agar using rabbit anti-rat serum, goat anti-rat IgG and an anti-rat L chain serum prepared in rabbits. A continuous gradient elution of the DE 52 column to 0·3 M phosphate, pH 7·8, failed to yield pure Fc.

The rat Fc was further purified by affinity chromatography. Pure rat L chains were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Incorporated) and specific anti-rat L chain antibodies were isolated from antisera. An anti-rat L chain–agarose column was similarly prepared and any residual Fab was removed from the Fc preparations.

Preparation of radiolabelled, monomeric IgG or IgG fragments. Each of the above whole IgG or IgG fragment preparations were labelled with ^{125}I by the iodine monochloride technique (Helmkamp *et al.*, 1960). The resultant iodinated proteins possessed one mole of iodine per mole of protein, with specific activities of 50,000 to 80,000 ct/min/μg. Monomeric preparations of IgG, Fc or Fab were obtained by gel filtration on Sephadex G-200 or G-100 after iodination. The aggregate-free Fab and Fc were of identical size, approximately 45,000 Daltons, as determined by the elution profiles on a calibrated Sephadex G-100 column. All of the protein preparations were more than 98% precipitable by 10% trichloroacetic acid (TCA) in the presence of serum or added albumin.

Serum disappearance studies. Female Wistar–Furth rats (Microbiological Associates, Bethesda, Maryland) or female Sprague–Dawley rats (Tyler Laboratories, Bellevue, Washington), weighing 200 g, were placed on KI-containing drinking water (0·6 g/l) for 3 days before use and also during the experiments. The radiolabelled proteins (20–100 μg in 0·5 ml) were injected into the saphenous vein after light ether anaesthesia. Blood samples (200 μl) were obtained from the tail of all animals at 5, 10, 15, and 30 min, 1, 2, 4, 8, and 12 hr after injection, then two or three times daily for 4 days after injection, except for nephrectomized animals which were sampled only through 52 hr. The sera were separated and 50-μl aliquots taken and mixed with 1 ml of normal saline. One millilitre of 20% TCA was added to each sample and the precipitates separated

and washed twice with cold 10% TCA. The washed precipitates were dissolved in 2 ml of 0·2 M NaOH. All samples were counted for 10 min in a well-type scintillation counter (Nuclear Chicago, Des Plaines, Illinois) equipped with a 2 × 3 inch sodium iodide crystal. Only protein-bound counts were used for the serum disappearance data.

The protein-bound counts present in the 5, 10, 15, and 30 min serum aliquots were plotted on a logarithmic scale against time on a linear scale. The ct/min present in the serum at the time of injection were obtained by visual linear extrapolation of this plot to zero time. The serum disappearance data points at each elapsed time were then expressed as a percentage of the ct/min present in the serum at the time of injection. The disappearance data were analysed by computer utilizing iterative curve fitting by the least squares method. In early studies, the SAAM 22 program of Berman & Weiss (1967) was utilized. In later studies, the less complex Box P program (Box & Lucas, 1959) was used after it was demonstrated that the two programs yielded identical data. In each study, estimated lambda (0·693/half-life in hours) and sigma (percentage of material possessing that $t_{1/2}$) values for two or three component disappearance curves were given to the computer. The number of curve components best fitting the experimental data points were determined for each separate animal. In addition, lambda and sigma values were obtained for each component.

Two to four animals were injected with each protein preparation and separate half-lives and sigmas calculated for each animal. The means and standard deviations were calculated from these data for each protein preparation.

Biological filtration experiments. Biologically filtered Fc was obtained by injecting one rat with 2·4 mg ^{125}I-labelled Fc. At 24 hr the animal was exanguinated by cardiac puncture. At that time 10% of the injected protein remained in circulation, which is comparable to the fraction remaining after injection with 100-fold less protein (see Results section). This suggests that the large amount of Fc injected did not saturate the catabolic mechanism. Aliquots (0·5 ml) of this serum containing the biologically filtered Fc were injected into normal rats, tail bleedings taken, and the serum disappearance curves calculated as described above.

Urine studies. Additional animals were injected with each of the above purified, radiolabelled proteins, using similar amounts as in the serum disappearance studies. The rats were kept in metabolic cages and 24-hr urine collections were made for 4 days. A small amount of toluene was added to each collection cup to suppress bacterial growth. Aliquots of each 24-hr urine sample were counted to determine total counts. Protein-bound counts in a 1-ml aliquot were determined by TCA precipitation after adding 0·1 ml of a 1 g/100 ml solution of rabbit albumin as a carrier protein. The total counts and protein bound counts in each daily urine sample were expressed as a percentage of the amount injected. Four animals were injected with each Fc and Fab preparation, and the means and standard deviations were calculated.

Sodium maleate pretreatment or nephrectomy. Previous studies have shown that sodium maleate blocks proximal tubule reabsorption of proteins from the glomerular filtrate. This property has been utilized to assess the role of the proximal tubule in the catabolism of light chains (Mogielnicki, Waldmann & Strober, 1971). Therefore, experiments were carried out in which an intraperitoneal injection of 200 mg/kg sodium maleate was given 8 hr prior to infusion of Fc or Fab. Serum and urine samples were obtained and analysed as described above for untreated animals. Separate groups of animals pretreated with sodium maleate were used for serum disappearance or for quantitative urine studies.

In another group of animals bilateral nephrectomy was performed by the abdominal approach under ether and light Nembutal anaesthesia. Two hours after the procedure an aliquot of Fc or Fab was injected into a saphenous vein. Serial blood samples were obtained from the tail and the serum disappearance curves were determined as outlined above. All animals remained alive for at least 52 hr after the bilateral nephrectomy.

Whole body catabolism. Known amounts of Fc preparations (in 0·5 ml) were given intravenously to normal and to nephrectomized rats. At intervals after injection (2, 6, 12, 24, 48 and 72 hr) the rats were killed with ether and residual urine was expressed from the bladder. The animals were then homogenized in a heavy duty Waring blender in 300 ml water. All tissues were well homogenized except for the skin, which remained in long strands. Five millilitre aliquots of the homogenates were mixed with 5 ml of 20% TCA, and the precipitates washed twice with 10% TCA. The ct/min in the washed TCA precipitates represented protein-bound counts in the sample. The total protein-bound counts remaining in the whole body at each time were calculated and expressed as a percentage of the amount injected. The mean and standard deviation (s.d.) was calculated for each time using data from two rats.

Autoradiography. Additional rats were injected intravenously with ^{125}I-labelled Fc, Fab or whole rat IgG, and killed at 5 min, 3 hr or 24 hr after injection. Approximately 500 μg and 30 million ct/min were injected into each animal. Immediately prior to killing, the animals were anaesthetized with ether and the left kidney was exposed through a midline abdominal incision. Karnovsky's fixative (Karnovsky, 1965) was poured into the abdominal cavity, and the exposed kidney was immersed for 15 min. The left kidney was excised and placed in the same fixative for an additional 4 hr at 4°C. A thin sliver of the outer cortex of the ventral renal surface was shaved off and the tissue washed overnight in 0·1 M sodium cacodylate with 7% sucrose. The tissue was subsequently soaked in 1% osmic acid buffered with 0·1 M s-collidine at pH 7·2, dehydrated

in a graded series of ethanol and propylene alcohol solutions and embedded in epoxy resin. One-micron thick sections mounted on glass slides were coated with Kodak NTB nuclear emulsion diluted 1:1 with distilled water. The autoradiographs were developed in Dektol after exposure of from 1–5 weeks, and then stained with Toluidine blue.

Miscellaneous. All rat protein concentrations were determined by absorbance at 280 nm using the following extinction coefficients ($E^{1\%}_{1\,cm}$) determined for rabbit immunoglobulin fragments: IgG 14·4; Fc 12·2; Fab 15·3; L chains 13·2 (Little & Donahue, 1968). Density gradient ultracentrifugation was carried out using 5–15% linear sucrose gradients centrifuged at 37,000 rev/min for 16 hr at 4°C (SW 41ti rotor and LK 50 preparative centrifuge, Beckman Division of Spinco Inc., Palo Alto, California). Significance testing was performed using Student's *t*-test.

RESULTS

Serum disappearance of rat IgG and Fc preparations

Serum disappearance experiments were carried out first with intact IgG and with Fc preparations. The IgG was removed from serum with a two component curve, exhibiting $t_{1/2}$ values of 2·18 and 49·3 hr (Table 1, Fig. 1). In contrast, the serum disappearance

TABLE 1. Serum disappearance of IgG and Fc fragment

Immunoglobulin preparation	Rats	Number of animals studied	Component	$t_{1/2}$ (hr) (Mean ± 1 s.d.)	Percentage (mean ± 1 s.d.)
IgG	Normal	4	First	2·18 ± 0·58	37·6 ± 6·6
			Second	49·3 ± 8·4	59·6 ± 7·4
Fc	Normal	4	First	0·248 ± 0·033*	30·9 ± 2·9
			Second	2·63 ± 0·54	55·5 ± 2·3
			Third	39·5 ± 7·9	13·9 ± 2·6
Fc (biologically-filtered)†	Normal	2	First	0·405, 0·194	21·3, 31·4
			Second	2·71, 2·52	55·0, 46·3
			Third	54·0, 43·0	24·3, 23·4
Fc	Maleate-treated‡	4	First	0·472 ± 0·084*	35·6 ± 3·6
			Second	2·85 ± 0·15	53·1 ± 3·8
			Third	49·5 ± 3·6	11·4 ± 0·6
Fc	Nephrectomized§	4	First	0·355 ± 0·160	27·9 ± 7·5
			Second	3·37 ± 0·36	54·5 ± 7·3
			Third	44·6 ± 6·6	17·0 ± 2·4

* Significantly different at $P < 0·005$ between Fc in normal and in maleate-treated rats.

† Biologically-filtered preparations were obtained by exsanguination of normal rats 24 hr after injection of Fc.

‡ Rats received an intraperitoneal injection of sodium maleate, 200 mg/kg, 8 hr before the infusion of Fc.

§ Rats infused with Fc 2 hr after bilateral nephrectomy.

curve of each Fc preparation was composed of three exponential components. The most rapid phase, not seen with IgG, had a $t_{1/2}$ of 0·25–0·38 hr (Table 1 and Fig. 1). The slowest or catabolic component of Fc serum disappearance was identical to that seen with IgG. Sucrose density gradient analyses on serial serum samples showed that IgG, Fc, and Fab fragments did not interact with serum proteins and that at all times the proteins in circulation remained aggregate-free.

Studies were performed with Fc preparations under different experimental conditions in an effort to further characterize the nature of the rapid removal of Fc from serum. Some Fc was biologically filtered to remove any molecules possibly denatured during preparation. The biologically filtered Fc showed serum disappearance characteristics identical to those

of the unfiltered preparation (Table 1). These results indicated that the rapid phase of Fc removal from serum was not due to selective uptake of denatured material by the mononuclear phagocyte system.

In order to examine the role of the kidneys in the clearance of Fc from serum, experiments were performed with Fc in rats pretreated with sodium maleate or after bilateral nephrectomy (Table 1, Fig. 2). Pretreatment with sodium maleate slightly prolonged the rate of the rapid phase of Fc serum disappearance. Removal of the kidneys, however, did not alter the serum disappearance curve. These results were compatible with two possibilities: (1) Fc fragments were not filtered in the kidneys, or (2) some Fc underwent glomerular filtration but in the absence of the kidneys Fc was removed from serum by other processes at a rate identical to that of glomerular filtration.

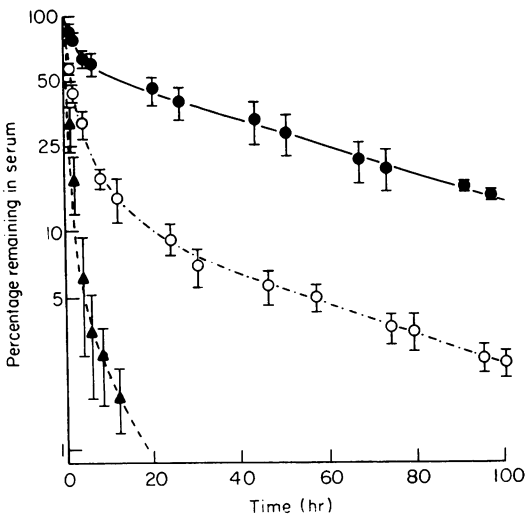

FIG. 1. Serum disappearance curves of rat IgG (●——●), monomeric Fc (○ - · - · - ○) and Fab (▲ – – ▲) in normal animals. The data are expressed as the percentage of injected material remaining in the serum *vs* time in hours. Each point represents the results obtained from four different rats, expressed as mean ± 1 s.d. Fab was rapidly removed from serum, while both Fc and IgG possessed a slow phase of serum removal with the same half-life.

Serum disappearance of rat Fab

The serum disappearance curve of Fab was markedly altered by nephrectomy, in contrast to the results seen with Fc. A two-phase serum disappearance curve was seen with Fab in normal rats, the two components possessing $t_{1/2}$ values of 0·25 hr and 1·7 hr (Table 2, Fig. 1). As with Fc, the $t_{1/2}$ of the first phase of Fab removal was slightly prolonged by pretreatment with sodium maleate. In nephrectomized rats, however, the rapid component of the Fab serum disappearance curve was no longer present. The disappearance curve now had three components. The $t_{1/2}$ of the first component was 1·31 hr, similar to the second component in intact animals (Table 2 and Fig. 2). There were also two new components of Fab serum disappearance with $t_{1/2}$ values of 4·63 and 14·4 hr. In all of these experiments the serum disappearance of Fab was followed until less than 1% of the injected material was remaining.

These results indicated that glomerular filtration was the major mechanism for the rapid removal of Fab from serum. In the absence of the kidneys the Fab fragments were equilibrated and catabolized in other organs at a much slower rate.

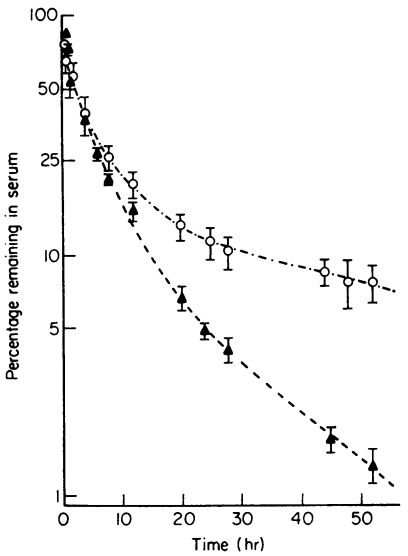

FIG. 2. Serum disappearance curves of Fc (○ – · – · – ○) and Fab (▲ – – ▲) in nephrectomized rats. The disappearance characteristics of Fc were unchanged in comparison with data obtained from normal animals (Fig. 1). The rapid component of Fab disappearance was no longer seen, although the curve was still not similar to that seen with Fc.

TABLE 2. Serum disappearance of Fab fragment

Immunoglobulin preparation	Rats	Number of animals studied	Component	$t_{1/2}$ (hr) (mean ± 1 s.d.)	Percentage (mean ± 1 s.d.)
Fab	Normal	4	First	0·247 ± 0·087*	61·3 ± 10·6
			Second	1·71 ± 0·25	39·2 ± 11·0
Fab	Maleate-treated	3	First	0·400 ± 0·008*	76·3 ± 0·5
			Second	1·83 ± 0·18	23·7 ± 0·7
Fab	Nephrectomized	4†	First	1·31 ± 0·19	45·6 ± 6·6
			Second	4·63 ± 1·01	36·8 ± 6·1
			Third	14·4 ± 0·5	15·8 ± 1·3

* Significantly different at $P < 0·05$ between Fab in normal and in maleate-treated rats.

† Analysis of the disappearance curves for these four experiments were done by the visual peeling method as there were too few data points for accurate computer analysis.

Metabolic clearance studies with Fab or Fc

After the injection of a radiolabelled protein into an animal, the rate at which free ^{125}I appears in the urine can serve as an estimate of the rate of catabolism (Waldmann & Strober, 1969). In order to study the relationship between serum disappearance and catabolism of Fab and Fc fragments, complete urine collections were made for 4 days following injection of each protein. The total counts in the urine in each 24-hr period and the protein-bound counts excreted in each 24-hr period were each expressed as a percentage of the total injected dose. Sucrose density gradient analyses on dialysed urine showed that all of the protein-bound counts represented excretion of intact Fc or Fab.

Experiments performed with Fc suggested that this protein was in part filtered in the kidney, reabsorbed and catabolized (Table 3). In the 1st day after administration of Fc to

normal rats, a total of 52·5% of the injected counts appeared in the urine with 6·02% of the injected dose excreted as protein-bound radioactivity. Pretreatment with sodium maleate did not change the total number of counts excreted in the 1st day but the protein-bound counts increased to 17·0% of the injected dose.

Analysis of the results of complete urine collections through the 4th day after injection of Fc failed to show uniform clearance characteristics suggestive of a single rate of catabolism. Non-linear plots of the daily excretion of free counts as a function of time were obtained. The daily excretion of free counts was expressed as a percentage of the specific activities of the intravascular, the extravascular, or the total body pool of remaining ^{125}I-labelled Fc. Identical results were obtained with biologically filtered Fc, indicating that any non-linearity in Fc catabolism was not due to the presence of denatured molecules or to inhomogeneity in the Fc preparations. These results suggested that Fc was not catabolized by a single process.

When Fab fragments were administered to normal rats, 90% of the total injected counts were excreted in the first day, with 14·6% of the injected dose appearing in the urine as protein-bound counts (Table 3). After pretreatment with sodium maleate the total urinary counts in the first day were unchanged but the protein-bound counts increased to 20·1% of the injected dose. The results of our studies indicated, therefore, that both Fab and Fc in the glomerular filtrate were subjected to uptake and probable degradation by proximal tubule cells.

Whole body catabolism of Fc

In order to further characterize the handling of Fc by the intact organism and to compare the kinetics of catabolism with those of serum disappearance, whole body catabolic experiments were performed with aggregate-free Fc.

When injected into normal rats Fc showed a two-component curve of whole body catabolism (Fig. 3). The two phases had $t_{1/2}$ values of approximately 6 and 32 hr. However, similar experiments in nephrectomized rats showed a loss of the rapid phase of whole body catabolism. The single component of Fc catabolism in nephrectomized rats had a

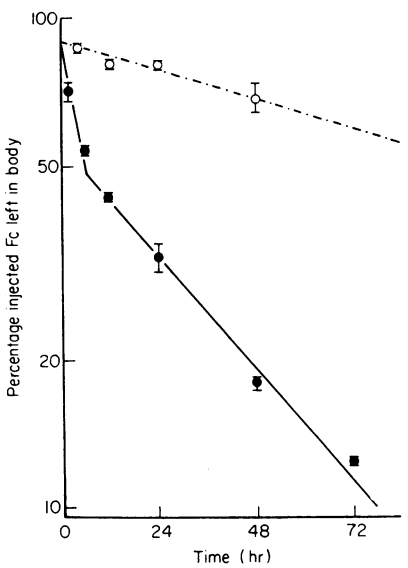

FIG. 3. Whole body catabolic curves of Fc in normal rats (●———●), and in nephrectomized rats (○ – · – · – ○). The catabolic curve of Fc in normal rats showed two components with the rapid phase of Fc catabolism no longer seen in nephrectomized rats.

TABLE 3. Metabolic clearance of Fc and Fab fragments

Immunoglobulin preparation	Number of animals studied	Day	Percentage of injected counts excreted per day* (mean ± 1 s.d.)			
			Total		Protein-bound	
			Normals	Maleate-treated	Normals	Maleate-treated
Fc	4	1	52·5 ± 6·1	54·1 ± 11·5	6·02 ± 1·58†	17·0 ± 1·90†
		2	13·7 ± 1·6	20·5 ± 8·3	0·81 ± 0·27	2·38 ± 1·22
		3	7·10 ± 1·69	6·42 ± 1·49	0·49 ± 0·09	0·50 ± 0·14
		4	3·43 ± 1·00	3·10 ± 0·28	0·26 ± 0·09	0·28 ± 0·04
Fab	4	1	90·0 ± 4·7	89·8 ± 4·3	14·6 ± 1·0‡	20·1 ± 3·9‡
		2	2·78 ± 0·18	6·29 ± 3·21	0·13 ± 0·02	0·25 ± 0·13
		3	0·49 ± 0·16	0·43 ± 0·08	< 0·1	< 0·1
		4	0·22 ± 0·11	0·17 ± 0·05	< 0·1	< 0·1

* The total counts (free iodine and protein-bound) appearing in the urine each day after injection, and the protein-bound counts only, are each expressed as a percentage of the total injected material.

† $P < 0·001$ between excretion of intact Fc in normal and in maleate-treated rats.

‡ $P < 0·02$ between excretion of intact Fab in normal and in maleate-treated rats.

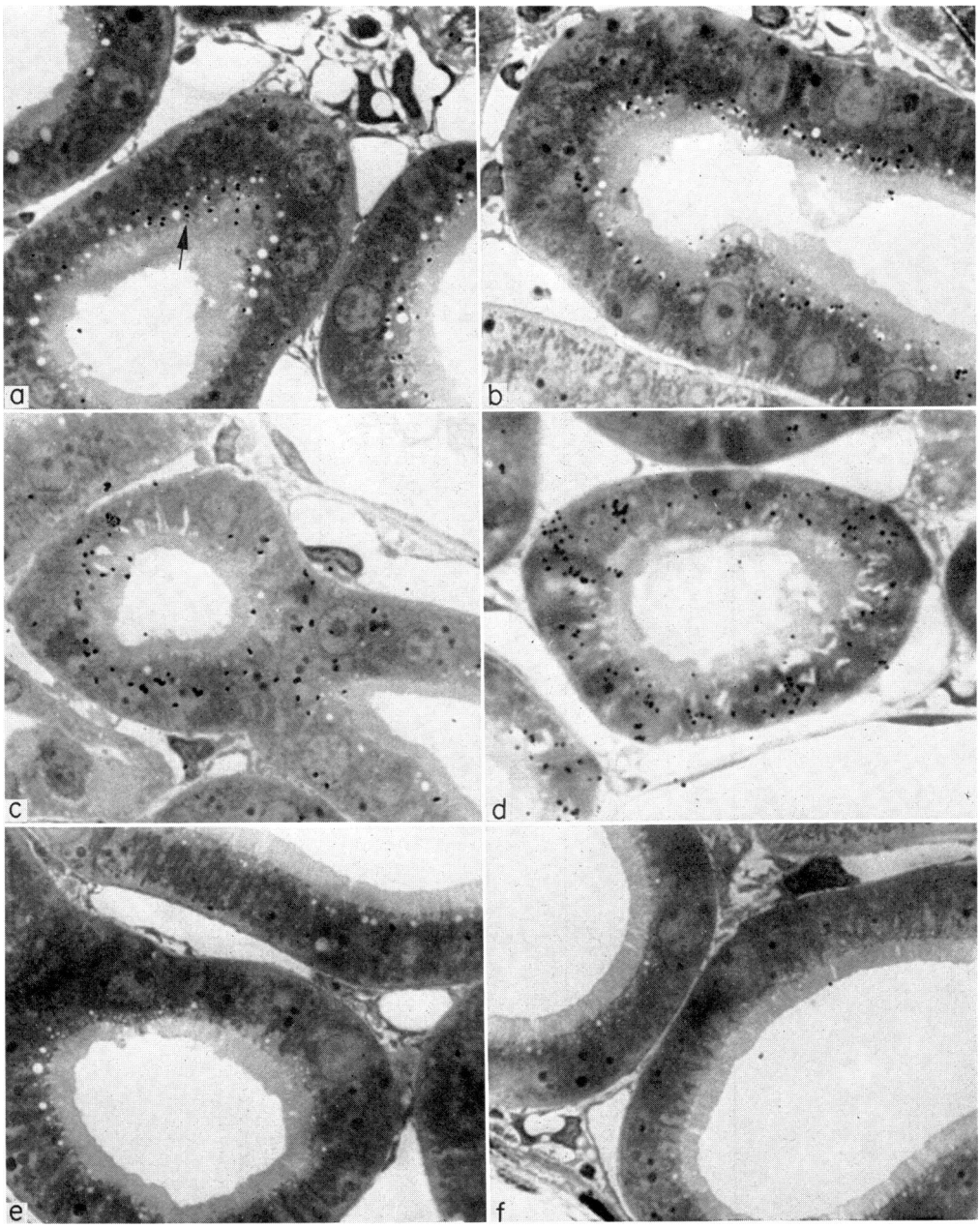

FIG. 4. Autoradiographs of epoxy sections stained with toluidine blue. (Original magnification $\times 1035$.) (a) Proximal tubule of rat which received ^{125}I-labelled Fc 5 min before sacrifice. Note the presence of silver grains overlying the apical cytoplasm (arrow). (b) Proximal tubule of rat which received ^{125}I-labelled Fab 5 min before sacrifice. The location of silver grains is similar to that seen in (a). (c) Proximal tubule of rat which received ^{125}I-labelled Fc 3 hr prior to sacrifice. Silver grains are distributed throughout the cytoplasm of the tubular cells. (d) Proximal tubule of rat which received ^{125}I-labelled Fab 3 hr prior to sacrifice. Localization of silver grains is similar to (c). (e) Proximal tubule of rat which received ^{125}I-labelled Fc 24 hr prior to sacrifice. No specific labelling is seen. (f) Proximal tubule of rat which received ^{125}I-labelled Fab 24 hr prior to sacrifice. As in (e) there is no specific labelling.

$t_{1/2}$ of approximately 105 hr. Identical results were obtained with biologically filtered Fc (results not shown).

These results indicated that some Fc fragments were subjected to renal catabolism, particularly in the first 6 hr after injection. The failure of the semi-logarithmic plots of percentage injected Fc left in the body as a function of time to extrapolate to 100% was due to technical reasons. The skin was poorly homogenized and significant protein-bound counts were adherent to skin at all times 2 hr or longer after injection.

Autoradiographic studies

The handling of Fc and Fab by the kidneys was further examined by autoradiography. Aliquots of radiolabelled proteins were injected into the saphenous vein and animals killed at 5 min, 3 and 24 hr (see Materials and Methods section). It is important to note that with *in vivo* kidney perfusion techniques, as utilized in these studies, little to no free iodine is left in the tissue (Maunsbach, 1966).

These studies provided further evidence that the kidneys participate in the catabolism of immunoglobulin fragments, but not that of intact IgG. Five minutes after the administration of Fab or Fc, radioactivity was localized in the apical cytoplasm of the proximal convoluted tubule cells (Fig. 4a and b). By 3 hr silver grains were distributed over the medial and basal cytoplasm (Fig. 4c and d). By 24 hr, no specific localization was observed (Fig. 4e and f). No structures other than proximal tubules were specifically labelled. In contrast to the results seen with immunoglobulin fragments, renal tissue from animals injected with IgG did not show specific activity at any of the times studied. The autoradiographic tissue sections in Fig. 4 represent development after different times of exposure. No attempt was made to quantitate the grain counts in these studies.

DISCUSSION

The data in this paper describe the serum disappearance characteristics, metabolic clearance, and whole body catabolism of homologous IgG fragments in rats. The results suggest that the mechanisms whereby Fc fragments are handled by the body differ from those involving Fab. Although the kidneys represented the major organ for filtration and catabolism of Fab fragments, a smaller proportion of injected Fc underwent renal catabolism. The major portion of Fc appeared to become rapidly equilibrated with an unidentified pool where it was not available for glomerular filtration.

These studies were primarily concerned with the early phases of serum disappearance of Fc and Fab fragments, representing filtration and equilibration. The size of a molecule is the major factor determining its susceptibility to filtration across basement membranes (Renkin & Gilmore, 1973). Other factors affecting filtration are the shape, electrical charge, and binding characteristics of the molecule (Heinemann, Maack & Sherman, 1974). The physical characteristics (size and shape) of a molecule which affect its relative susceptibility to glomerular filtration are the same characteristics which determine its elution profile by gel filtration chromatography (Renkin & Gilmore, 1973). In our experiments the elution profiles of Fab and Fc on a calibrated Sephadex G-100 column were identical, corresponding to a molecular weight of 45,000 Daltons. If glomerular filtration were the major mechanism for the rapid removal from serum for both Fab and Fc then the equilibration phases of the serum disappearance curves should have been identical for the two proteins, or at least similar. The persistence of an unaltered rapid component of Fc serum disappearance in nephrectomized rats suggests that in addition to filtration based on size other properties of the Fc molecule such as the binding characteristics or electrical charge influenced its fate immediately after injection. The results of recent experiments indicated that the charge of the Fc molecule was not important in influencing its rapid disappearance

from serum. Ovalbumin, which has a mol. wt of 45,000 Daltons and carries a net negative charge, showed serum disappearance characteristics similar to those seen with the positively charged Fab fragment (unpublished observations). Fc, which carries an intermediate charge, showed serum disappearance characteristics markedly different from either Fab or ovalbumin.

Therefore, we suggest that the specific binding characteristics of Fc influenced its fate in the body in the time immediately after injection. The results of our studies do not, however, demonstrate the anatomical localization of Fc fragments or the mechanism of rapid removal from serum in the absence of the kidneys. The Fc molecules did not appear to be selectively adherent to cells in circulation or to platelets (unpublished observations). Sucrose density gradient analyses on serum samples obtained at serial times after injection failed to provide any evidence of strong interaction of a major portion of the injected Fc or Fab with serum proteins. However, the Fc may have been weakly adherent to the first component of complement, Clq, with dissociation occurring under the conditions of ultracentrifugation. The possibility remains that the injected Fc rapidly established an equilibrium with specific surface receptors on such cells as marginated leucocytes, endothelial cells, or tissue phagocytic cells, and was made unavailable for filtration in the kidneys. Organ localization studies at 1 hr after injection failed to identify the site of this hypothetical compartment. Both Fab and Fc were located diffusely throughout the body with the exception of the kidneys and urine where 11·3% of injected Fc and 35·2% of the Fab were found at 1 hr (unpublished observations). Further studies are necessary to determine the cellular, and subcellular, localization of the injected Fc and the possible relevance of the mechanisms of handling of Fc to IgG catabolism.

In addition to examining the phase of rapid disappearance from serum, our experiments confirm and extend previous studies on the catabolism of IgG fragments. The disappearance of Fab from serum was markedly altered by nephrectomy. In the absence of the kidneys, Fab equilibrated with extravascular pools and the terminal phase of Fab serum disappearance had a $t_{1/2}$ of 14·4 hr. Wochner *et al.* (1967) obtained similar results for the half-life of whole body catabolism of rabbit Fab in nephrectomized mice. In contrast to Fab, the serum disappearance of Fc was not altered by nephrectomy. There are three lines of evidence, however, indicating that some injected Fc underwent renal filtration and catabolism. First, inhibition of proximal tubular protein reabsorption by maleate treatment increased the amount of intact Fc excreted in the 1st day after injection from 6% of the injected dose in normal rats to 17%. Second, nephrectomy eliminated the rapid phase of whole body catabolism of injected Fc. Third, autoradiographic studies localized Fc to the proximal tubule, the major site of protein reabsorption and catabolism in the kidney (Maunsbach, 1966).

The results of experiments in nephrectomized rats showed that the $t_{1/2}$ of Fc whole body catabolism (Fig. 3) was longer than the third or slowest component of the Fc serum disappearance curve (Fig. 2, Table 1). This apparent discrepancy may have been due to the inability to obtain data points beyond 52 hr in the nephrectomized rats, possibly leading to a falsely fast $t_{1/2}$ for the terminal phase of Fc serum disappearance. The prolongation of the rate of whole body catabolism of Fc in nephrectomized rats probably was due in part to the effects of uremia (Wochner *et al.*, 1967).

The whole body catabolic curve of homologous rat Fc in our studies showed two phases, with the rapid component being eliminated by nephrectomy. Studies by Wochner *et al.* (1967) showed that the whole body catabolic curve of rabbit Fc in mice possessed only a single component which was not significantly altered by nephrectomy. The reasons for this difference are not apparent but could be related to species variations in the size, or other characteristics, of Fc fragments prepared in the presence of reducing agents. Previous studies indicated that the excretion of intact Fc in the first 3 days after injection varied between 1·9% and 18·8% of the injected dose, utilizing various heterologous or homologous com-

binations of Fc and experimental animals (Spiegelberg & Weigle, 1965). The excretion of intact Fc, however, was greater for preparations obtained after 18 hr of papain digestion than for molecules subjected to only 5 min of papain digestion. Further experiments showed that prolonged exposure to reducing agents, such as cysteine or β-mercaptoethanol, led to Fc preparations which showed a greater percentage of material removed from serum in the rapid component (Spiegelberg & Weigle, 1965; Spiegelberg & Fishkin, 1972). Thus, it is possible that different periods of time of exposure to papain or to reducing agents, as well as species variations in the effect of cysteine on Fc structure lead to Fc preparations which are filtered to varying degrees in the kidneys during the equilibration period.

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