Clin. exp. Immunol. (1978) 32, 207–217.

The influence of phagocyte function on glomerular localization of aggregated IgM in rats

A. KIJLSTRA, ALLEGONDA VAN DER LELIJ, D. W. KNUTSON, G. J. FLEUREN & L. A. VAN ES
Department of Nephrology, University Hospital, Leiden, and Department of Pathology, University of Groningen, Groningen, The Netherlands

(*Received* 19 *July* 1977)

SUMMARY

The blood clearance and hepatic localization of aggregated human IgM (AIgM) were studied in relation to the glomerular localization patterns in rats. AIgM was cleared rapidly from the circulation by sinusoidal cells in the liver, monomeric IgM (MIgM) was cleared less rapidly. Localization of AIgM within the glomerulus was dependent on the level in the blood. Low doses led only to a mesangial localization, whereas high doses resulted in a localization along the capillary walls. Immunoelectron microscopy showed that in the glomerular capillaries the AIgM was localized within endothelial cells or subendothelially, and did not occur within the glomerular basement membrane. Inhibition of the hepatic uptake of AIgM by colloidal carbon resulted in increased levels of circulating AIgM and prolonged the deposition of AIgM in the glomerular capillaries.

INTRODUCTION

It is now widely accepted that antigen–antibody complexes are responsible for the development of vasculitis and glomerulonephritis in both man and experimental animals (Cochrane & Koffler, 1974). However, immune complexes can be present in the circulation without leading to glomerulonephritis (Bayer *et al.*, 1976). When immune complexes formed *in vitro* are injected into animals, their localization in glomeruli is variable and inconsistent (Cochrane, 1971). Where glomerular deposition has been demonstrated, the localization was usually mesangial (Okumura, Kondo & Tada, 1971; Haakenstad, Striker & Mannik, 1976). The factors thought to play a role in the glomerular localization of immune aggregates include the size of the particles (Cochrane & Hawkins, 1968), the composition of the complex (Lightfoot, Drusin & Christian, 1970; Mannik & Arend, 1971; Haakenstad & Mannik, 1976) and the dose (Haakenstad & Mannik, 1974), as well as the vascular permeability (Cochrane & Hawkins, 1968; Kniker & Cochrane, 1968; Benveniste, Henson & Cochrane, 1972) and RES function (Haakenstad & Mannik, 1974; Wardle, 1974; Morgan & Steward, 1976; Ford, 1975).

The study reported here was undertaken to determine the effect of decreased phagocyte function on the clearance rate and tissue localization of immune aggregates. Human IgM was chosen because it has a sedimentation rate of 19S in its monomeric form, which is thought to be the critical size for the deposition of immune complexes in tissues (Cochrane & Hawkins, 1968). Phagocyte function was impaired by the administration of colloidal carbon prior to the injection of immune aggregates. The results show that a glomerular localization of immune aggregates is dependent on the levels in the circulating blood, which in turn are dependent on phagocyte activity.

MATERIALS AND METHODS

Aggregated IgM. IgM was isolated from the serum of a patient with Waldenström's macroglobulinaemia by boric acid precipitation (van Breda Vriesman & Feldman, 1972), followed by gel filtration with Biogel A5 (Bio-Rad Laboratories,

Correspondence: Dr A. Kijlstra, Department of Nephrology, University Hospital, Leiden, The Netherlands.

0099-9104/78/0500-0207$02·00

Richmond, California). The MIgM obtained from several isolations was pooled, pressure-concentrated to 10 mg/ml and stored at −20°C in 0·1 M borate-buffered saline (BBS) at pH 7·4. The same MIgM pool was used for all experiments. Protein concentrations were determined by the Folin method or by absorbance at 280 nm, using an extinction coefficient ($E_1^{1\%}_{cm}$) of 13·5, IgM was iodinated with ^{125}I to a specific activity of approximately 1 μCi/mg by the chloramine T method (McConahey & Dixon, 1966). TCA precipitability was 95% or more at the time of use. Aggregated ^{125}I-labelled IgM (^{125}I-labelled AIgM) was prepared by heating ^{125}I-labelled IgM (10 mg/ml) at 63°C for 30 min. The degree of aggregation was assessed by sucrose density gradient centrifugation, using linear gradients of 10–30% w/v sucrose in BBS. Aggregates were centrifuged in a Spinco SW-41 rotor for 4 hr at 263,000 *g*.

Carbon. Rats were injected i.v. with 1 ml of a colloidal carbon suspension (Günther Wagner 11/1431a), which was centrifuged before use to remove large aggregates and contained 100 mg carbon per ml. The same carbon solution was used in all experiments. Clearance of carbon from the circulation was measured on the basis of optical density at 650 nm of timed blood samples (Biozzi, Benacerraff & Halpern, 1953). AIgM clearance and localization were determined 4 hr (group AC4) and 18 hr (group AC18) after carbon administration.

Experimental protocol. Experiments were performed on ether-anaesthetized male inbred Wistar rats raised in our institution and weighing 145–155 g. Groups of twenty-four rats, either untreated or pre-treated with colloidal carbon, received 10 mg ^{125}I-labelled AIgM or ^{125}I-labelled MIgM via the penile vein. Organ and blood localizations were investigated in sets of four rats killed by exsanguination 15, 30, 60, 120, 180 and 240 min after the injection. Specific organ localization was calculated after correction for the residual blood volume (RBV), which was determined by i.v. injection of homologous ^{59}Fe-labelled red blood cells (Sharple, Culbreth & Klein, 1950) 10 min before death. Immediately after death, the organs (lungs, kidney, spleen and liver) were removed and weighed, after which a small slice of each organ was snap-frozen for immunofluorescence studies and the remainder was re-weighed and the ^{125}I and ^{59}Fe activity determined. In addition, a 50 μl blood sample was taken and added to 1·0 ml 0·1% sodium carbonate for the determination of ^{125}I and ^{59}Fe activity, after which 1 ml 10% TCA was added and the precipitate counted. All samples were counted in a Packard triple-channel well counter. ^{125}I counts in each sample were corrected for the crossover of ^{59}Fe in the ^{125}I channel. The amount of IgM in the blood and organs was calculated as a percentage of the injected dose and expressed per total blood volume and per total organ, as follows:

$$\text{total blood volume (ml)} = \frac{^{59}\text{Fe ct/min injected}}{^{59}\text{Fe ct/min per ml blood}};$$

$$\text{and the percentage of injected dose in total blood volume} = \frac{(\text{ml total blood volume} \times {}^{125}\text{I ct/min per ml blood})}{^{125}\text{I ct/min injected}} \times 100.$$

The ^{125}I ct/min in the blood and the ^{125}I ct/min injected represent the TCA-precipitable counts.

$$\mu\text{l RBV per total organ} = \frac{^{59}\text{Fe ct/min per total organ}}{^{59}\text{Fe ct/min per } \mu\text{l blood}};$$

and the percentage of specific organ localization of injected dose =

$$\left(\frac{^{125}\text{I ct/min per total organ} - \mu\text{l RBV per total organ} \times {}^{125}\text{I ct/min per } \mu\text{l blood}}{^{125}\text{I ct/min injected}}\right) \times 100.$$

The ^{125}I ct/min in the blood is given by the counts obtained before the TCA precipitation, and the ^{125}I ct/min injected by the TCA-precipitated counts.

The recovery of the injected dose was calculated by adding the percentage of total organ localization of all organs investigated to the percentage in the total blood volume. Since catabolic products are known to disappear rapidly from phagocytic cells (Shinomiya & Koyama, 1976), the serial decrease in the total recovery of TCA-precipitable ^{125}I in the blood and specific ^{125}I localization in the organs under study was used as a measure of catabolism.

Immunofluorescence. Localization of IgM was studied qualitatively with the immunofluorescence technique. Small slices of each organ were snap-frozen in isopentane immediately after removal. Cryostat sections were cut 2 μm thick and stained for human IgM by the indirect technique with the use of a rabbit anti-human IgM antiserum and a fluorescein-conjugated IgG preparation of goat anti-rabbit IgG. Staining for rat C3 was done by the direct technique with a goat anti-rat C3 (Nordic, Tilburg, The Netherlands). Tissue sections were examined in a Leitz–Orthoplan microscope equipped with an epi-illuminator provided with an Osram HBO 100 W mercury lamp, a BG 38 red-absorbing filter, 2 × KP 490 narrow band-passing filters and a S525 barrier filter. Photographs were taken on Kodak Tri-X Pan Film, ASA 400 (Eastman Kodak Co., Rochester, New York).

Electron microscopy. IgM was detected in renal tissue by immunoelectron microscopy using the unlabelled antibody enzyme method (Sternberger, 1974). Small pieces of renal cortex were fixed in a formaldehyde–glutaraldehyde mixture (Karnovsky, 1965) during 5 min at room temperature. Subsequently the tissues were rinsed in Tris–maleic acid buffer (pH 7·38) for 2 hr at room temperature, snap-frozen in pre-cooled Freon (−100°C) and cut in a cryostat. The 20 μm cryostat sections were transferred to a small vial for incubation with the antisera at room temperature for 30 min. Each incubation was followed by a rinse in PBS (pH 7·2) for 30 min. In sequence, the serum layers consisted of rabbit anti-human IgM (1 : 16), goat anti-rabbit antibody (1 : 16) and rabbit anti-peroxidase (1·25 mg/ml). The 'super-sandwich' was completed by the enzyme horseradish peroxidase in a concentration of 0·0375 mg/ml. Following the incubations and washing proce-

dures, the horseradish peroxidase was reacted with a 75 mg/100 ml solution of 3,3'-diaminobenzidine tetrahydrochloride to which 0·001% hydrogen peroxide was added, for 3 min at room temperature. After a short rinse in PBS, the sections were post-fixed in 0·1 M phosphate-buffered 2% OsO_4 for 45 min at room temperature and subsequently embedded in Epon 812. Ultra-thin sections were cut on a LKB Ultrotome III and examined unstained in a Philips EM-300 electron microscope.

Complement fixation. Serum was obtained from rats before and at various times after the i.v. injection of AIgM (10 mg), MIgM (10 mg) or BBS alone. Complement fixation which had occurred *in vivo* was then assessed by measuring the total haemolytic complement (CH_{50}) (Kabat & Mayer, 1967).

RESULTS

IgM aggregation

Heating of ^{125}I-labelled IgM solutions at 63°C for 30 min at a concentration of 10 mg/ml resulted in a polydispersed population of soluble aggregates, as shown by sucrose density gradient centrifugation (Fig. 1). Approximately 90% of the ^{125}I-labelled AIgM was in an aggregated form. The sedimentation rate of the aggregates ranged from 19S to 120S.

The sucrose density gradient profile did not change when AIgM was incubated in normal rat serum for 4 hr at 37°C (data not shown).

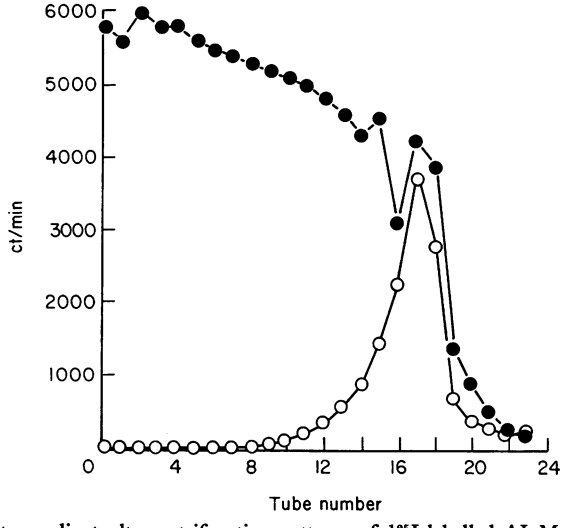

FIG. 1. Sucrose density gradient ultracentrifugation patterns of ^{125}I-labelled AIgM (●) and MIgM (○). The top of the gradient is at the right.

Complement fixation

Complement fixation *in vivo* was seen during the first 10 min after injection of MIgM (Table 1). Later values for MIgM and all but one value (20 min) for AIgM did not differ from those obtained with BBS. Increased vascular permeability for either AIgM or MIgM was not found, as judged from the haematocrit values; histamine was injected as a positive control (Table 2). No complement fixation was observed in kidney sections when C3 localization was studied by immunofluorescence. These kidney sections, which were prepared 1 hr after an i.v. injection of 20 mg AIgM, showed granular deposits of AIgM along the capillary loops and in the mesangium.

Blood clearance and organ localization of ^{125}I-labelled AIgM and ^{125}I-labelled MIgM

The clearance and tissue localization of IgM were compared in groups of rats given 10 mg of either ^{125}I-labelled AIgM or ^{125}I-labelled MIgM (Figs 2 and 3). AIgM (group A) was cleared from the circulation more rapidly than MIgM (group M). This was correlated with an increased liver localization of AIgM as compared with animals given MIgM (Figs 2 and 3). Localization in the spleen, kidneys and lungs was very low and did not exceed 1·1% of the injected dose per total organ. More AIgM than

TABLE 1. Complement fixation at various intervals after i.v. injection of MIgM, AIgM or BBS*

Time (min)	MIgM†	AIgM‡	BBS§
0	100%	100%	100%
5	78·2±10·6¶	85·0±9·5	94·0±6·2
10	77·1± 7·2¶	84·6±6·7	91·1±6·6
20	75·1±11·8	78·7±4·2¶	90·1±6·3
30	74·6±12·3	78·9±2·3	86·0±6·2

* Data are expressed as the percentage of CH_{50} units (± 1 s.d.) at $t = 0$.

† Four rats given 10 mg MIgM.

‡ Four rats given 10 mg AIgM.

§ Four rats given 1 ml freshly prepared BBS.

¶ Significantly different from BBS (Student's t-test; $P < 0{\cdot}05$).

TABLE 2. Haematocrit changes at various intervals after injection of histamine, AIgM, MIgM or BBS*

Time (min)	BBS†	10 mg histamine‡	AIgM§	MIgM¶
0				
5	95·2±4·7	114·6±2·6	89·2±2·6	91·2±3·3
10	95·3±5·6	117·8±2·6	89·6±3·0	92·8±1·8
15	93·8±5·0	115·2±2·6	88·9±2·3	90·7±2·4
45	93·0±4·6	98·1±2·2	88·7±2·7	88·4±3·4

* Data expressed as percentage of haematocrit at $t = 0$; mean values of four rats ± 1 s.d.

† Rats given 1 ml BBS.

‡ Rats given 10 mg histamine.

§ Rats given 10 mg AIgM.

¶ Rats given 10 mg MIgM.

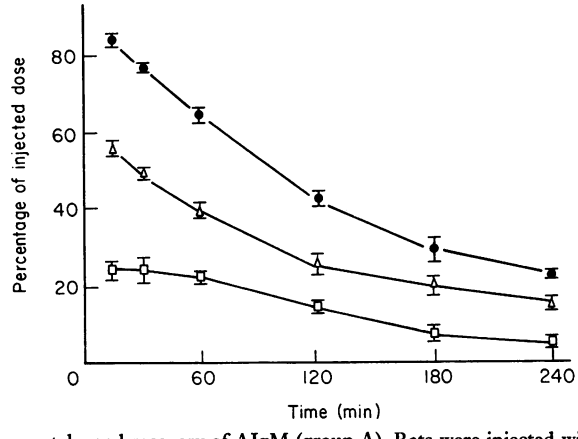

FIG. 2. Clearance, liver uptake and recovery of AIgM (group A). Rats were injected with 10 mg ^{125}I-labelled AIgM. Blood clearance (△) is expressed as the percentage of the injected dose per total blood volume, the liver uptake (□) as the percentage of the injected dose per total liver corrected for residual blood volume and recovery (●) as the sum of the radioactivity in the blood, liver, lungs, kidneys and spleen. Each point represents the mean value of four rats (± 1 s.d.).

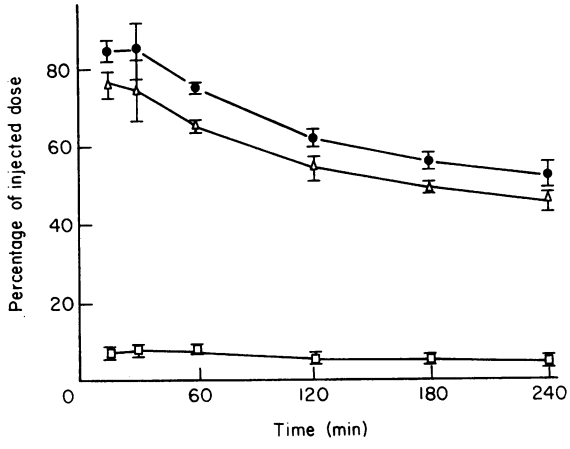

FIG. 3. Clearance, liver uptake and recovery of MIgM (group M). Rats were injected with 10 mg ^{125}I-labelled MIgM. Blood clearance (△) liver uptake (□) and recovery (●) are expressed as indicated in Fig. 2.

MIgM was seen only in the spleen. The summation of radioactivity in the total blood and whole organs shows a more rapid decrease of the recovery of AIgM than of MIgM, which indicates a higher catabolism of AIgM (Figs 2 and 3).

Immunofluorescence microscopy showed that in the liver AIgM was deposited exclusively in the cells lining the sinusoids, presumably Kupffer cells. The fluorescence was very bright shortly after the injection (15–120 min) and became less pronounced in the period between 180 and 240 min.

The kidney localization of AIgM was investigated by immunofluorescence after injection of various doses of AIgM (1–20 mg). In a separate experiment the influence of dose on blood clearance was assessed during the first 60 min (Fig. 4). An increase in the dose led to higher blood levels of AIgM. Clearance constants were identical for all doses applied, thus indicating first-order clearance kinetics. The renal localization of AIgM was both time- and dose-dependent (Table 3). No IgM, or only a trace, was found when 1 mg AIgM was given, and after doses of 2 and 4 mg deposition occurred only in the glomerular mesangium. At higher doses (10 and 20 mg) AIgM was initially (15–120 min) seen in a granular pattern in the walls of the glomerular and peritubular capillaries and in the mesangium (Fig. 5).

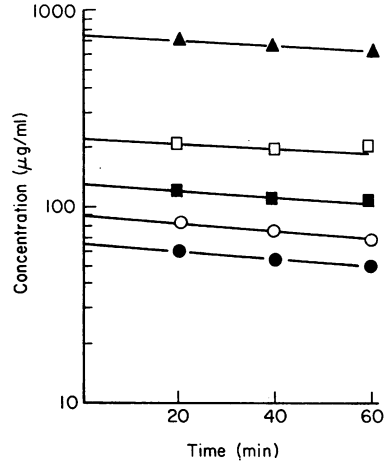

FIG. 4. Clearance of AIgM from the circulation. Rats (n = 4) were injected with various doses (1–10 mg) of ^{125}I-labelled AIgM. Clearance is expressed as TCA-precipitable radioactivity per ml blood. Doses of ^{125}I-labelled AIgM: (●) 1·0 mg, (○) 1·3 mg, (■) 2·0 mg, (□) 3·3 mg and (▲) 10 mg.

TABLE 3. The influence of dose and time on the localization pattern of AIgM in the rat glomerulus

Dose (mg)	Number of rats	Time after injection of AIgM (hr)				
		½	1	2	3	4
1	2		Neg.*	Neg.	Neg.	Neg.
2	4		M	M	M	M
4	2		M			M
10	4	M†–C‡	M–C	M	M	M
20	2		M–C	M–C	M	M

* Neg. = negative to trace amounts in mesnagium.
† M = mesangial localization.
‡ C = capillary localization.

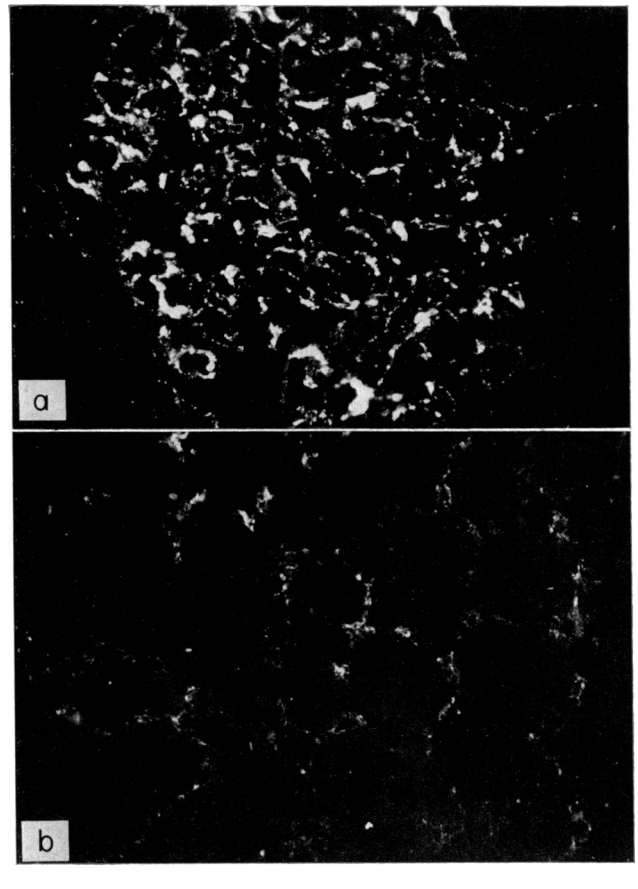

FIG. 5. Immunofluorescent pattern of IgM in a rat kidney 1 hr after injection of 20 mg AIgM. Granular fluorescence is seen along the walls of glomerular (a) and peritubular capillaries (b). (Magnification: (a) × 560; (b) × 280.)

Kidneys collected at later times (2–4 hr) showed an arborized deposition of IgM in the mesangium (Fig. 6), whereas the capillaries showed negative or trace deposition. Comparison of the effects of the 10 and 20 mg doses shows that the higher dose caused prolonged glomerular and peritubular capillary staining (Table 3). An injection of MIgM (10 mg) did not result in renal localization.

To determine the localization of AIgM within the capillary wall, immunoelectron microscopy was performed with peroxidase as a label. As Fig. 7 shows, AIgM was found within endothelial cells and also subendothelially. No staining was seen within the glomerular basement membrane.

Effect of colloidal carbon. A biphasic disappearance of carbon from the circulation was found after i.v. injection of 1 ml of a suspension containing 100 mg colloidal carbon. The half-life ($T\frac{1}{2}$) of the first phase was 1·5 hr, and that of the second phase was 3 hr. No circulating carbon was detected after 11 hr.

FIG. 6. Immunofluorescence pattern of IgM in a rat glomerulus 4 hr after injection of 20 mg AIgM. Aggregates are seen mainly within the mesangium. (Magnification × 560.)

AIgM (10 mg) injected either 4 hr (group AC4) (Fig. 8) or 18 hr (group AC18) (Fig. 9) after carbon administration showed large differences in both the disappearance rates and the liver localization. In the AC4 group, the AIgM clearance was slower and resembled that of MIgM (group M) in untreated rats. In the AC18 group, the clearance was initially faster and then quite comparable to the rate observed in animals not given carbon. The hepatic level was constant in the AC4 group, but became very high in the AC18 group. The recovery data (Figs 8 and 9) show that carbon-treated animals catabolized AIgM more slowly than untreated animals. Immunofluorescence studies on tissue collected 15–240 min after the injection of 10 mg AIgM revealed that the granular staining of AIgM in the glomerular and peritubular capillaries persisted longer in the AC4 group than in group A. The fluorescence was still present in both areas at the end of the longest interval studied (4 hr) (Fig. 10). When AIgM was injected 18 hr after carbon injection, glomerular capillary staining did not persist longer in group AC18 than in group A.

DISCUSSION

In this study the disappearance rate and phagocytic uptake of AIgM were investigated in relation to its deposition and persistence in the walls of renal capillaries. AIgM injected into rats disappeared rapidly from the circulation and localized mainly in the liver, where it is normally catabolized. In the kidney a granular pattern developed along the walls of glomerular and peritubular capillaries soon after the injection, and then gradually changed into a predominantly mesangial localization. Immunoelectron microscopy showed that the glomerular capillary localization was subendothelial and within endothelial cells; AIgM was not seen in the glomerular basement membrane. MIgM did not appear in the glomeruli, despite the slower clearance from the circulation leading to higher blood levels. Our findings are in agreement with those obtained by Michael, Fish & Good (1967) and Mauer *et al.* (1972), who showed

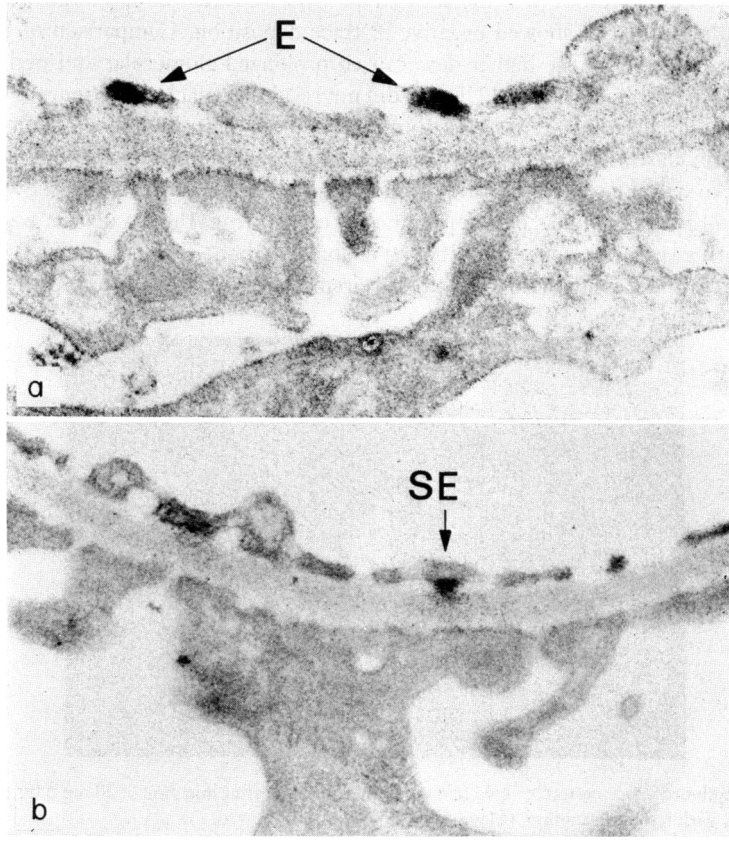

FIG. 7. Immunoelectron micrograph made 1 hr after the injection of 20 mg AIgM. (a) Localization of AIgM within endothelial cells (E); (b) localization of AIgM in a subendothelial position (SE). (Magnification × 26,000).

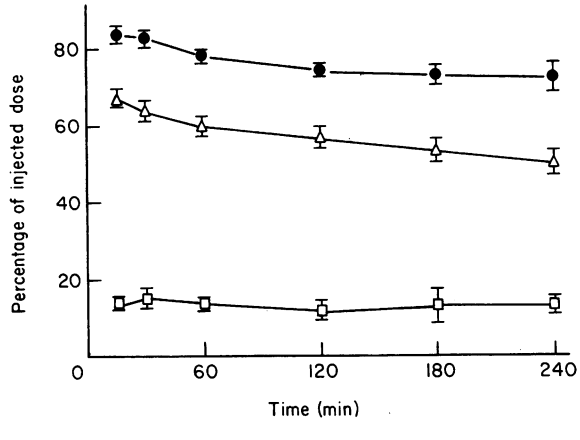

FIG. 8. Clearance, liver uptake and recovery of aggregated IgM in rats given 10 mg ^{125}I-labelled AIgM 4 hr after the injection of 100 mg colloidal carbon (group AC4). Blood clearance (△) liver uptake (□) and recovery (●) are expressed as indicated in Fig. 2.

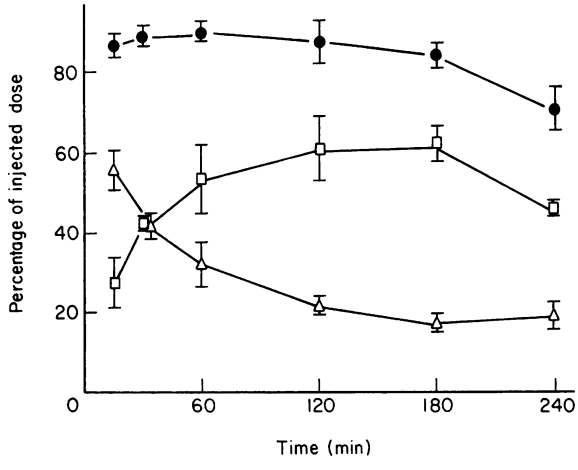

FIG. 9. Clearance, liver uptake and recovery of aggregated IgM in rats given 10 mg ^{125}I-labelled AIgM 18 hr after the injection of 100 mg colloidal carbon (group AC18). Blood clearance (△) liver uptake (□) and recovery (●) are expressed as indicated in Fig. 2.

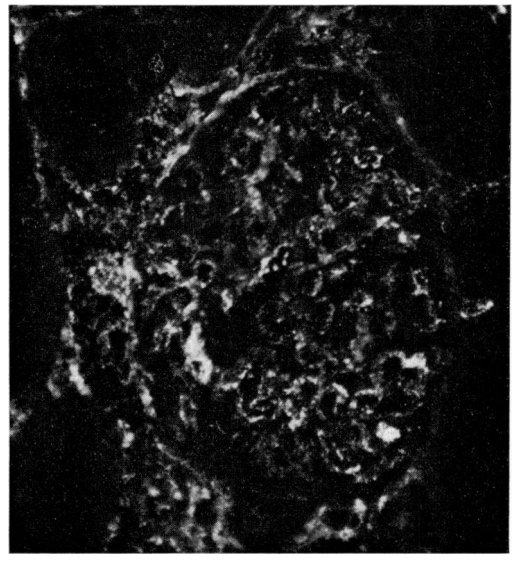

FIG. 10. Immunofluorescence pattern of IgM in a glomerulus 4 hr after injection of 10 mg AIgM into a rat pre-treated with carbon (group AC4). The aggregates are mainly localized in the glomerular and peritubular capillaries. (Magnification × 352.)

that aggregation is necessary for the glomerular deposition of both BSA and IgG. Furthermore, the renal localization pattern found by these authors is the same as that seen in our study. Our results show that the persistence of AIgM in renal capillaries is dependent on the AIgM blood level, which is influenced by the dose given and the degree of uptake by phagocytes in the liver.

The liver has been shown to be largely responsible for the removal of aggregated proteins (Mauer *et al.*, 1972) and immune complexes (Benacerraf, Sebestyen & Cooper, 1959; Haakenstad & Mannik, 1974). In our study, AIgM was found mainly in the liver, other organs showing very small amounts.

The predominant clearance and catabolism of AIgM by the liver is further supported by the accumulation of AIgM in the liver observed in rats treated with carbon 18 hr earlier. In contrast, when AIgM was given 4 hr after carbon, clearance was slow, and accumulation in the liver did not occur. The

total recovery of AIgM in both carbon-treated groups was very high, which indicates that catabolism was decreased.

The most likely explanation for these observations is that carbon has two effects on the function of phagocytic cells in the liver (Normann, 1970). Soon after administration (4 hr), carbon inhibits the liver uptake of AIgM, as was shown by Normann (1974) for other aggregated proteins. When AIgM is injected after carbon has disappeared from the circulation (18 hr), there is no competition and binding is restored. However, due to the intracellular presence of carbon, the phagocytic cells are unable to process the AIgM, which leads to a large hepatic accumulation of AIgM and a high recovery. Localization of AIgM in glomerular capillaries was prolonged when the removal of AIgM from the circulation was inhibited by a prior injection of carbon (group AC4). However, AIgM staining was not prolonged when carbon pretreatment did not elevate the blood level of AIgM (group AC18). Thus the inhibited uptake by the phagocytes in the liver, leading to elevated AIgM blood levels, probably resulted in the continued deposition of AIgM in the glomerular and peritubular capillaries.

This observation was also made by Ford (1975), using immune complexes in carbon pre-treated animals, but he does not mention the influence of carbon pre-treatment on circulating immune complex levels.

Various authors have shown that apart from the primary blocking effect on phagocytes, carbon injection also causes a marked thrombocytopenia in experimental animals (van Aken & Vreeken, 1970; Elema, Hoyer & Vernier, 1976). Whether this secondary effect of carbon pre-treatment was responsible for the prolonged capillary localization of AIgM in rats of the AC-4 group seems unlikely because: (a) prolonged capillary localization was not observed in animals given immune aggregates 18 hr after carbon pre-treatment, whereas according to Elema *et al.* (1976) the thrombocytopenia induced with this dose is comparable at 4 hr or 18 hr after carbon injection; (b) vascular permeability was not increased in group AC4 compared to control experiments, as measured by haematocrit values (A. Kijlstra, unpublished observations). High blood levels of AIgM, either induced by injecting high doses of AIgM in normal animals (Table 3) or by decreasing the clearance of AIgM from the circulation by carbon injection thus determine the renal capillary localization of AIgM.

It remains possible that the prolonged deposition of AIgM in the glomeruli was caused by an impaired clearing mechanism due to the presence of carbon in the mesangium (Elema *et al.*, 1976). This seems unlikely, because the capillary localization of AIgM was not prolonged when carbon was given 18 hr earlier; according to Elema *et al.* (1976) carbon is still present at this time. In most passive immune complex models, glomerular deposition was restricted to the mesangium (McCluskey *et al.*, 1960; Okumara *et al.*, 1971; Haakenstad *et al.*, 1976). Capillary deposition occurred in passive models only after the injection of large doses of aggregated IgG (Mauer *et al.*, 1972; Michael *et al.*, 1967) or after the injection of immune complexes into 'carbon-blocked' animals (Ford, 1975). Immunoelectron microscopy performed in this study, and ultrastructural studies done by Michael *et al.* (1967), showed that the capillary deposits were localized not in the glomerular basement membrane, but subendothelially and endothelially. The localization of pre-formed immune complexes within the glomerular basement membrane seen in active immune complex models in rabbits (Andres *et al.*, 1963) has not yet been demonstrated in passive immune complex models. The mechanism responsible for the intramembranous deposition of immune complexes therefore remains obscure.

This study was supported by a grant from the Netherlands Kidney Foundation, grant No. C72, and was presented in part at the 1975 meeting of the Dutch Society of Nephrology (abstract: *Kidney Int.* **9**, 449).

We are indebted to Dr J. G. Eernisse for providing us with the serum of a patient with Waldenström's macroglobulinaemia. We thank Mrs Hella van Welij for her assistance in the preparation of the manuscript.

REFERENCES

Aken, W.G. van & Vreeken, J. (1970) The clearance mechanism of the reticuloendothelial system. *Mononuclear Phagocytes* (ed. R. van Furth), p. 382. Blackwell Scientific Publications, Oxford.

Andres, G.A., Seegal, B.C., Hsu, K.C., Rothenberg, M.S. & Chapeau, M.L. (1963) Electron microscopic studies of experimental nephritis with ferritin-conjugated antibody. Localization of antigen–antibody complexes in rabbit

glomeruli following repeated injections of bovine serum albumin. *J. exp. Med.* **117**, 691.

BAYER, A.S., THEOFILOPOULOS, A.M., EISENBERG, R., DIXON, F.J. & GUZE, L.B. (1976) Circulating immune complexes in infective endocarditis. *New Engl. J. Med.* **295**, 1500.

BENACERRAF, B., SEBESTYEN, M. & COOPER, N.S. (1959) The clearance of antigen-antibody complexes from the blood by the reticuloendothelial system. *J. Immunol.* **82**, 131.

BENVENISTE, J., HENSON, P.M. & COCHRANE, C.G. (1972) Leukocyte-dependent histamine release from rabbit platelets: the role of IgE, basophils and platelet activating factor. *J. exp. Med.* **136**, 1356.

BIOZZI, G., BENACERRAF, B. & HALPERN, B.N. (1953) Quantitative study of the granulopectic activity of the reticuloendothelial system. II. *Brit. J. exp. Path.* **34**, 441

BREDA VRIESMAN, P.J.C. VAN & FELDMAN, J.D. (1972) Rat γM immunoglobulin: isolation and some biological characteristics. *Immunochemistry*, **9**, 525.

COCHRANE, C.G. (1971) Mechanisms involved in the deposition of immune complexes in tissues. *J. exp. Med.* **134**, Suppl., 75.

COCHRANE, C.G. & HAWKINS, D. (1968) Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localize in blood vessels. *J. exp. Med.* **127**, 137.

COCRHANE, C.G. & KOFFLER, D. (1974) Immune complex disease in experimental animals and man. *Advances in Immunology* (eds F. J. Dixon and J. H. Humphrey), **16**, 185. Academic Press, New York.

ELEMA, J.D., HOYER, J.R. & VERNIER, R.L. (1976) The glomerular mesangium: uptake and transport of intravenously injected colloidal carbon in rats. *Kidney Int.* **9**, 395.

FORD, P.M. (1975) The effect of manipulation of the reticuloendothelial system activity on glomerular deposition of aggregated protein and immune complexes in two different strains of mice. *Brit. J. exp. Path.* **56**, 523.

HAAKENSTAD, A.O. & MANNIK, M. (1974) Saturation of the reticuloendothelial system with soluble immune complexes. *J. Immunol.* **112**, 1939.

HAAKENSTAD, A.O. & MANNIK, M. (1976) The disappearance kinetics of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. *Lab. Invest.* **35**, 283.

HAAKENSTAD, A.O., STRIKER, G.E. & MANNIK, M. (1976) The glomerular deposition of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. *Lab. Invest.* **35**, 293.

KABAT, E.A. & MAYER, M.M. (1967) *Experimental Immunochemistry*, p. 135. Charles Thomas, Publisher, Springfield. Illinois, U.S.A.

KNIKER, W.T. & COCHRANE, C.G. (1968) The localization of circulating immune complexes in experimental serum sickness. The role of vasoactive amines and hydrodynamic forces. *J. exp. Med.* **127**, 119.

LIGHTFOOT, R.W., DRUSIN, R.E. & CHRISTIAN, C.L. (1970) Properties of soluble immune complexes. *J. Immunol.* **105**, 1493.

MANNIK, M. & AREND, W.P. (1971) Fate of pre-formed immune complexes in rabbits and rhesus monkeys. *J. exp. Med.* **134**, Suppl., 19.

MAUER, S.M., FISH, A.J., BLAU, E.B. & MICHAEL, A.F. (1972) The glomerular mesangium. I. Kinetic studies of macromolecular uptake in normal and nephrotic rats. *J. clin. Invest.* **51**, 1092.

MCCLUSKEY, R.T., BENACERRAF, B., POTTER, J.L. & MILLER, F. (1960) The pathologic effects of intravenously administered soluble antigen-antibody complexes. *J. exp. Med.* **111**, 181.

MCCONAHEY, P.J. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy appl. Immunol.* **29**, 185.

MICHAEL, A.F., FISH, A.J. & GOOD, R.A. (1967) Glomerular localization and transport of aggregated proteins in mice. *Lab. Invest.* **17**, 14.

MORGAN, A.G. & STEWARD, M.W. (1976) Macrophage clearance function and immune complex disease in New Zealand black/white F_1 hybrid mice. *Clin. exp. Immunol.* **26**, 133.

NORMANN, S.J. (1970) Function of the reticuloendothelial system. Evidence for two types of particle induced reticuloendothelial paralysis. *Infec. Immunity*, **1**, 327.

NORMANN, S.J. (1974) Kinetics of phagocytosis. II. Analysis of *in vivo* clearance with demonstration of competitive inhibition between similar and dissimilar foreign particles. *Lab. Invest.* **31**, 161.

OKUMURA, K., KONDO, Y. & TADA, T. (1971) Studies on passive serum sickness. I. The glomerulus fine structure of serum sickness nephritis induced by preformed antigen-antibody complexes in the mouse. *Lab. Invest.* **24**, 383.

SHARPLE, L.M., CULBRETH, G.C. & KLEIN, J.R. (1950) Blood and packed cell volume of the adult rat as measured by tagged cells. *Proc. Soc. exp. Biol. Med.* **74**, 681.

SHINOMIYA, T. & KOYAMA, J. (1976) *In vitro* uptake and digestion of immune complexes containing guinea-pig IgG1 and IgG2 antibodies by macrophages. *Immunology*, **30**, 267.

STERNBERGER, L.A. (1974) *Immunocytochemistry* (ed. by A. Osler and L. Weiss), p. 1. Prentice-Hall, Englewood Cliffs, U.S.A.

WARDLE, E.N. (1974) Reticuloendothelial clearance studies in the course of horse serum induced nephritis. *Brit. J. exp. Path.* **55**, 149.