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Quantitative elution studies in experimental immune complex and nephrotoxic nephritis

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SUMMARY

Optimal conditions have been determined for elution of antiglomerular basement-membrane antibodies and antigen–antibody complexes from nephritic kidneys, obtained from rabbits with nephrotoxic nephritis and various forms of BSA-induced immune complex disease. Elution from kidney homogenates was performed with acid and alkaline buffers, solutions of chaotropes, urea, sodium and magnesium chloride and other agents. Functional activity of antibodies exposed to elution procedures was tested by immunofluorescence, or by a modified Farr's technique. Antibodies of progressively greater binding capacity were eluted by a stepwise acid gradient (pH 3·2–2·8) using low or high ionic strength glycine–HCl buffer. Antigen–antibody complexes were best eluted using 3 M KBr (pH 9) using several extractive steps. A stepwise alkaline gradient (pH 10·5–11·1 using 0·1 M glycine–NaOH buffer) or 8 M urea were found to be satisfactory alternative methods of eluting immune complexes. Elution procedures were best carried out in the cold in all circumstances. Other eluting agents were found to be less successful or less practical.

INTRODUCTION

Allergic glomerular injury in experimental glomerulonephritis in animals can be mediated by antibodies against glomerular basement-membrane antigens or by glomerular deposition of antigen–antibody complexes (Germuth & Rodriguez, 1973; Dixon, Feldman & Vasquez, 1961). Evidence for involvement of these mechanisms in the pathogenesis of human glomerulonephritis has in part been based on elution studies of diseased kidneys. Antibodies eluted from patients suffering from anti-GBM disease have induced nephritis in primates (Lerner, Glassock & Dixon, 1967; McPhaul & Dixon, 1970); likewise, in a few cases of immune complex nephritis in man putative antigens have been identified *in situ* (Levy & Hong, 1973; Strauss *et al.*, 1975; Couser *et al.*, 1974) after specific antibodies have been eluted from kidney preparations. Acid buffers of low ionic strength (Lerner *et al.*, 1967; Gamble & Reardan, 1975), high ionic strength (Gallo, 1970; Koffler *et al.*, 1971), or chaotropic ions (Strauss *et al.*, 1975) have been the main dissociating agents utilised to elute antibodies. However, experiments to determine optimal conditions for elution of antibodies or antigens have not been reported in detail.

In order to examine conditions for eluting immune complexes or antibodies from kidneys we have induced in rabbits either a nephrotoxic nephritis or an acute serum sickness by the intravenous administration of radiolabelled antigen or antibodies. Diverse agents that have been proved useful in dissolution of immune precipitates (Kabat & Mayer, 1961; Ternynck & Avrameas, 1971), dissociation of antigens (Wood, Stephen & Smith, 1968) or antibodies (Dandliker & Saussure, 1968) from immunoadsorbents, or solubilization of glomerular basement membrane (Kefalides & Winzler, 1966; Marquardt, Wilson & Dixon, 1973) and other membranes (Penefsky & Tzagoloff, 1971; Dierich & Reisfeld, 1975) were tested against renal cortex homogenates. The influence of temperature, pH, molarity, time and

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eluting volume on yield was studied as was the functional integrity of antibodies after exposure to these conditions.

MATERIALS AND METHODS

Animals. 2·0–2·5 kg male New Zealand white rabbits were used. Animals were fed on pellet diet, drinking water containing sodium chloride and potassium iodide and kept in metabolic cages. Rabbits were killed with sodium pentobarbitone (Nembutal, Abbott).

Preparation of radiolabelled proteins. Crystallized bovine serum albumin (BSA) was purchased from Armour, U.K. Rabbit anti-BSA Ab was purified by affinity chromatography (Fuchs & Sela, 1973) from immune (batch A) and hyperimmune (batch B) rabbit sera raised against BSA after 4 and 9 weeks of immunization. Rabbit serum albumin (RSA) was prepared from rabbit serum by precipitation with acid and ethanol (Weigle, 1961). Rabbit globulin, sheep normal and sheep nephrotoxic-globulin (NTG) fractions were prepared by 50% saturated ammonium sulphate (SAS) precipitation (Heide & Schwick, 1973) from rabbit serum, sheep serum and nephrotoxic serum respectively. Rabbit globulin fraction and rabbit anti-BSA antibodies were ultracentrifuged at 143,000 *g* for 90 min prior to radioiodination. Protein concentration was measured by the method of Lowry *et al.* (1951). Radiolabelling was carried out with ^{125}I or ^{131}I by the chloramine-T method (McCohaney & Dixon, 1966).

Experimental animal models. One-shot acute serum sickness (ASS). Five rabbits were preimmunized with 3 mg of BSA in FCA by the i.m. route at day −5 followed on day 0 by 250 mg/kg body weight ^{125}I-labelled BSA i.v. ^{131}I-labelled RSA was administered (as a paired label marker) at least 24 h before the onset of immune elimination. Rabbits were killed when 99% immune elimination had occurred.

Two-shot ASS (three rabbits). ASS was induced as described above. When approximately 90% immune elimination of the first dose of BSA had occurred a second dose of 250 mg/kg body weight was given i.v. together with ^{131}I-labelled RSA. Animals were killed when 99% immune elimination of the second dose of BSA had occurred.

Two-shot ASS plus passive transfer of radioiodinated anti-BSA antibody (three rabbits). The following protocol was followed: (a) rabbits were preimmunized as described; (b) 250 mg/kg body weight of unlabelled BSA was given as the first dose; (c) when proteinuria appeared, a second dose of BSA radiolabelled with a low tracing dose of ^{131}I was administered; (d) between 30 and 100 mg of ^{125}I-labelled anti-BSA antibody protein, having a sp. act. of 400–600 ct/min per μg of protein were given i.v. on the day of 99% immune elimination of ^{131}I-labelled BSA; (e) a marker dose of ^{131}I-labelled rabbit normal globulin was administered conjointly; (f) animals were killed 24–48 h later and kidneys were processed and counted immediately and again when a parallel reference sample of ^{131}I-labelled BSA had decayed to background values.

Kidney-bound anti-GBM antibodies source. Ten rabbits were injected intravenously with 1·5 ml/kg body weight of ^{125}I-labelled NTG [containing ^{131}I-labelled normal sheep globulin as a paired marker (Thomson, 1976) and having a sp. act. of about 500 ct/min per μg of protein.] Animals were killed 24 h after injection.

Immune-elimination of circulating radiolabelled BSA and quantitation of circulating immune complexes. The rate of ^{125}I-labelled-BSA elimination from blood and the presence of immune complexes was determined by counting serum precipitates in 10% TCA and washed precipitates formed in 50%-saturated ammonium sulphate respectively.

Proteinuria. Proteinuria was qualitatively assessed by adding aliquots of 20% TCA to centrifuged urine samples.

Kidneys. Kidneys were perfused *in situ* with cold PBS, removed and kept at −70°C until further use.

Histological and immunofluorescent methods. These were as previously described (Evans *et al.*, 1973).

Renal bound ^{125}I-labelled BSA, ^{125}I-labelled anti-BSA and ^{125}I-labelled sheep nephrotoxic antibody. Renal-bound radiolabelled proteins were determined in washed renal cortex homogenates. Cortex was separated from medulla, minced and homogenized in a Waring Blender for 2 min at high speed in 5 vol. of cold phosphate-buffered saline (PBS). Homogenates were repeatedly washed in cold PBS until no radioactivity from the labelled RSA marker was detectable. After renal-bound radiolabelled protein had been assessed for each rabbit, homogenates were pooled, mixed thoroughly and divided into aliquots.

Eluting agents. All solutions were prepared from AR grade chemicals (BDH, U.K.).

Acid and alkaline buffers. Glycine–hydrochloric acid buffer was prepared at 0·1, 0·02 and 0·5 M in a pH range from 3·4 to 2·6; 0·1 and 0·02 M buffers were used in 20 vol. and 0·5 M buffer in 10 vol. per 1 vol. of homogenate. Glycine–sodium hydroxide was made at 0·1 and 0·02 M from pH 9 to 12 and used in a proportion of 10 vol. per 1 vol. of kidney (pH was measured at 20° C (using a PYE Model 292 pH meter provided with a glass and combined electrodes).

Chaotropic ions. 3 and 10 M potassium bromide and sodium thiocyanate were prepared in 0·05 M phosphate buffer (pH 5–8) and in 0·02 M glycine–sodium hydroxide (pH 9–10·5).

Other electrolytes. 3 M magnesium chloride and 2 M sodium chloride were prepared in 0·01 M phosphate buffer, pH 7·3.

Urea. 4 and 8 M deionized urea was prepared in 0·01 M phosphate buffer (pH 7·3) and in 0·1 M glycine–sodium hydroxide (pH 9 and 10·5).

Other agents. 0·1 M EDTA (pH 7·3), distilled water (pH 7), 1% sodium dodecyl sulphate in PBS, and BSA (1 mg/ml and 50 mg/ml) in PBS were also employed. Samples eluted in PBS represented the controls in each group of experiments.

Eluting procedures. Aliquots of washed homogenate of renal cortex containing radiolabelled antigen or antibody were incubated with eluting agents under constant magnetic stirring. The time, temperature, pH, molarity and volume used are

stated in the results section. The post-incubation mixtures were centrifuged at 20,000 *g* for 15 min at 4°C except for those containing chaotropic ions which were spun at 40,000 *g* for 60 min. The supernatants were neutralized immediately after centrifugation with 1 M dipotassium hydrogen orthophosphate or 0·5 M hydrochloric acid. Molarity was corrected by adding 2 M sodium chloride or by extensive dialysis in the cold against PBS. The dialysate was counted, then centrifuged and the pellet counted. The percentage of eluted counts was calculated. Non-protein-bound radioactivity was determined in aliquots from eluates taken before and after dialysis by precipitation with 20% TCA using normal rabbit serum as carrier. In all instances free radioactive iodine fluctuated between 0 and 1%.

The precipitate formed after dialysis of eluates is largely insoluble kidney protein. When chaotropes, urea or alkaline solutions were used to extract kidneys the precipitates after dialysis contained 10–25% of the original radioactivity. Since this material probably included precipitated complexes between extracted antigen and antibody, and the supernatants contained both antigen and antibody, undue reliance could not be placed on direct measurement of antigen-binding capacity (ABC) of eluted antibody.

The direct effect of eluting agents on antibody activity. The direct effect of eluting agents on antibody activity was therefore determined, using ^{131}I-labelled rabbit anti-BSA antibody (600 ct/min/μg). 50 μl aliquots containing 50 μg of antibody were incubated with 3 ml of eluting agent with or without the addition of 0·3 g of normal rabbit-kidney homogenate. The ABC of the antibodies was determined (after adjustment of molarity and pH) by a modified Farr technique (Minden & Farr, 1973). ^{125}I-labelled BSA having a specific activity of 450,000 ct/min per μg was used as antigen. Non-TCA-precipitable radioactivity was less than 2%. Triplicate aliquots of 500 μl were pipetted into 3 ml plastic tubes, counted and incubated with 4 μg of labelled BSA prepared in 500 μl aliquots of NRS (diluted 1/00); control tubes contained 'acid' eluates from normal rabbit kidneys. All tubes were left over-night at 4°C, counted, mixed with 1 ml of cold 100% SAS, centrifuged and the precipitates formed washed twice in 50% SAS and counted. The amount of non-antibody-bound ^{125}I-labelled BSA precipitated was calculated and subtracted from the total precipitate. Final results were calculated as the number of μg of labelled BSA combined to 1 μg of labelled antibody. Triplicate values differed from the mean by less than 10% in all cases. Acid extraction does not solubilize GBM antigen and though 5–15% of the original nephrotoxic antibody was lost in the precipitate forming after dialysis it was felt possible to test the ABC of the eluted antibodies. This was done semi-quantitatively using standard indirect immunofluorescence techniques. The dialysed supernatants from eluates were serially diluted in 1/10 normal rabbit serum (NRS) and incubated with 4 μm acetone-fixed cryostat sections of normal rabbit kidney for 30 min. After washing in PBS, these were then stained with rabbit FITC–anti-sheep immunoglobulin diluted 1/40 in 1/10 NRS. Intensity of fluorescent linear staining along glomerular and tubular basement membranes was scored from 0 to +++ and scores compared for comparable concentrations of antibody. Acid eluates from acute BSA nephritis were used as negative controls.

RESULTS

General

One-shot ASS. All five rabbits showed evidence of glomerulonephritis by histology and immunofluorescence and four of the five animals had proteinuria. The mean amount of renal-bound ^{125}I-labelled BSA was 20 μg (range 12–37) per total kidney weight (TKW).

Two-shot ASS. All three rabbits developed histological and immunofluorescent evidence of nephritis and proteinuria. The average amount of renal-bound BSA was found to be 320 μg (range 200–438) per TKW.

Two-shot ASS plus passive transfer of ^{125}I-labelled Ab. All three animals developed proteinuria with a mean value of renal-bound anti-BSA of 155 μg per TKW (range 30–230).

Nephrotoxic nephritis. All ten rabbits developed proteinuria with typical linear deposits of sheep immunoglobulin and rabbit C3 along the GBM and variable polymorphonuclear infiltration. 4830 μg of kidney-bound NTG were found in a pool of kidney homogenates from ten animals (equivalent to 241 μg per kidney).

Dissociation studies under acid conditions (Tables 1 and 2)

The results of elution of anti-GBM antibodies in 0·1 M glycine–MCl buffer after 1 hr incubation at 20°C are shown in Table 1. Similar yields were obtained at 4°C and at 37°C and in all cases gradual decrements in pH produced distinct increments in yield. More intense immunofluorescence, presumably indicating a better preservation of antigen-binding capacity, was observed when elution procedures were performed at 4°C (an average gain of 1 + on the fluorescence score). At 37°C fluorescence scores were 1 + lower than at 20°C.

A two-step elution at pH 3·0 and 2·8 at 4°C each for 60 min yielded about 60% of bound antibody.

TABLE 1. Yield and functional activity (by immunofluorescence) of anti-GBM antibodies eluted at varying (acid) pH at 20°C

	pH				
	3·4	3·2	3·0	2·8	2·6
Yield (%)	18	33	45	48	57
GBM*	n.d.	++	++	++	+
TBM	n.d.	++	+++	+++	++

n.d. = not done.

* GBM and TBM: binding of eluted antibody to GBM (glomerular basement membrane) and to TBM (tubular basement membrane) assessed by indirect immunofluorescence; result expressed 0 to +++.

Elution of anti-BSA antibodies of 'immune' type (batch A, see Methods) was readily achieved by 0·1 M acid buffers. With the two-step method, at pH 3·0 and 2·8 at 4°C for 1 hr each, 80 and 6% recovery was achieved (direct measurements of ABC of antibodies eluted showed higher values for antibody eluted at pH 2·8 (ABC = 3) than at pH 3·0 (ABC = 1). With antibody of 'hyperimmune' type (batch B, see Methods) only 10% was recovered with 0·1 M buffers. Higher yields were obtained with 0·02 M buffer (17%) and 0·5 M buffer (24%).

The effect of acid buffer on ABC of anti-BSA Ab (batch B) was assessed after incubation with buffers of varying molarity and pH (Table 2). Antibodies exposed at 37°C had a lower ABC than those at 4°C,

TABLE 2. Effect of eluting agents on ABC

Agent	Molarity	pH	ABC (4°C) (%)*	ABC (37°C) (%)*
NaSCN	3	4·5	89	57
		6·0	98	83
		7·4	100	71
		7·4†	97	51
		9·0	91	63
		10·5	86	43
KBr	3	9·0	96	79
		9·0†	91	64
		10·5	86	71
$MgCl_2$	3	7·3	89	91
NaCl	2	7·3	92	91
PBS	0·15	7·3	100	96
Gly–HCl	0·02, 0·1, 0·5	2·6	83	65
	0·1	3·0	95	85
	0·1	3·2	95	82
	0·02	3·2	96	85
Gly–NaOH	0·1	10·5	100	87
		11·1	95	73
		12·0	83	50
Urea	4	9·0; 10·5	100	83
	8	9·0; 10·5	95	75
Distilled water		7·0	87	61

* Percent of initial value.

† Incubated with kidney tissue.

the difference being more pronounced at the lower pH values. The presence or absence of kidney homogenate made no significant difference to the results obtained.

Elution of radiolabelled BSA by acid buffers was ineffective : 0·1 M glycine–HCl buffer (pH 3·0–2·6) eluted from 0–10% at 4°C or 3–12% at 37°C after a 1 hr incubation; and when 0·5 M buffer (pH 2·6) was used between 5 and 23% of BSA was obtained.

Elution under alkaline conditions (Fig. 1 and Table 2)

Fig. 1 illustrates the yield of radio-iodinated BSA or antibody to BSA from homogenates using 0·1 M glycine–sodium hydroxide under varying conditions of time, temperature and pH. The elution of antigen and Ab was found to be rapid and relatively time-independent. With single extractions, higher yields were obtained at 37°C than at 4°C but a three-step extraction, each for 20 min at 4°C, was the best.

The effect of alkaline buffers on the ABC of anti-BSA Ab is shown in Table 2. ABC was well preserved at low temperature at pH 10·5–11·1; the presence of kidney tissue in the incubation mixtures had no effect.

Dissociation studies with chaotropic ions

Elution of anti-GBM antibodies. Using sodium thiocyanate solutions as eluate (Fig. 2) it was found that only slight increases of yield were obtained by increasing incubation time, incubating at 37°C

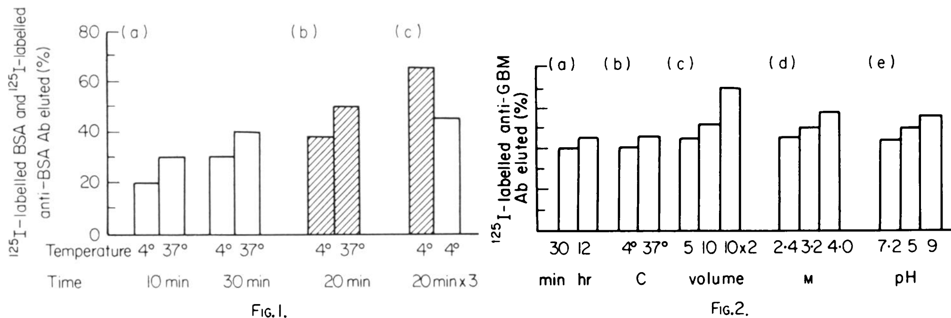

FIG. 1. Elution of radiolabelled BSA (open columns) and anti-BSA Ab (batch B) (hatched columns) under alkaline conditions. (a) pH 11·1, (b) pH 10·5, and (c) three extractive steps—pH 10·5, 10·8 and 11·1. The times and temperatures used are shown on the abscissa.

FIG. 2. Effect of varying time (a), temperature (b), volume—parts of solution per 1 part of homogenate (c), molarity (d), and pH (e), on elution of anti-GBM antibcdy by sodium thiocyanate.

instead of 4°C or using the more concentrated solution. Yields of single extraction were higher at a moderately alkaline or acid pH and with larger volumes of solution. Two half-hour extractions with ten volumes of 3 M potassium thiocyanate at pH 7·4 extracted 70% of antibody.

Elution of BSA and anti-BSA Ab. Fig. 3 compares the elution of both radio-iodinated BSA and anti-BSA from renal homogenates at 4°C and at 37°C in the presence of either 2·5 M sodium thiocyanate (pH 6·5) or 2·4 M potassium bromide (pH 9). Homogenates were incubated in ten parts of solution for 30 min. Procedures at 37°C resulted in only slightly greater yields than those at 4°C; potassium bromide solution was more effective than thiocyanate.

In Table 2 is shown the effect of pH, temperature and kidney tissue on the ABC values of anti-BSA antibodies preincubated in solutions containing chaotropes. Values obtained at 37°C were lower than those at 4°C; furthermore, this loss of ABC was enhanced in the presence of kidney tissue, and was more marked with sodium thiocyanate than potasium bromide.

Effect of concentrated urea solutions (Fig. 4)

Antigen was poorly eluted (8%) in 4 M urea at pH 7·3; higher yields were observed when urea

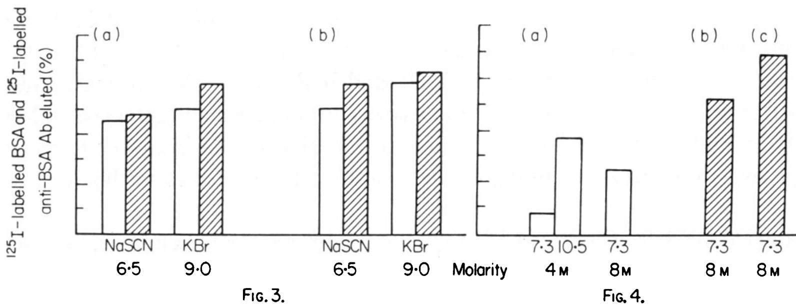

FIG. 3. Elution with chaotropes of radiolabelled BSA (open columns) and anti-BSA Ab (batch B) (hatched columns), (a) at 4°C, (b) at 37°C.

FIG. 4. Elution of radiolabelled BSA (open columns and anti-BSA Ab (batch B) (hatched columns) using 4 M or 8 M urea under various conditions, (a) 30 min at 37°C, (b) 30 min at 4°C, and (c) 30 min × 2 at 4°C.

concentration was doubled (25%) or when experiments were performed at pH 10·5 (37%) but similar yields had been obtained at pH 11·1 without urea (see Fig. 1a).

High yields (*c* 50%) of antibody were obtained with 8 M urea at neutral pH especially after two extractive steps. ABC values of anti-BSA antibodies preincubated in high-molarity urea solutions (at alkaline pH) are shown in Table 2. ABC values from antibodies treated at 4°C were similar to those obtained with PBS.

Dissociation studies with magnesium chloride and sodium chloride

Elution studies were undertaken with 2·5 M magnesium chloride and 2 M sodium chloride at neutral pH for 30 min (at 37°C). With the former 15% of BSA and 18% of anti-BSA and with the latter 6% and 10% respectively were dissociated from kidney homogenates. Similar ABC values (Table 2) were obtained when anti-BSA antibodies were preincubated in magnesium chloride, sodium chloride or PBS (at 37°C or 4°C).

Trials with other agents

Distilled water (pH 7, 20 vol.) eluted about 25% of anti-BSA antibodies (batch A) from renal homogenates after 60 min at 4°C. ABC values (Table 2) of Ab preincubated in DW were similar to those obtained at pH 2·6.

Neither 0·1 M EDTA (pH 7·3) nor BSA (1 mg/ml) eluted significant amounts of labelled BSA from kidney homogenates (containing 10 μg of BSA) after 30 min of incubation at 37°C. When similar aliquots were incubated for 75 min (at 37°C) with 50 mg of BSA, 28% of antigen was eluted (PBS control eluted 5·4%).

Sodium dodecyl sulphate (1% in PBS) eluted 48% of BSA (58% in the presence of 4 M urea in PBS) when kidney homogenate was incubated for 30 min at 37°C.

DISCUSSION

The aim of these studies was to determine optimal conditions of elution of antibodies and immune complexes from kidneys using experimental animal models, with the object of applying these techniques to the study of human nephritis. No comprehensive investigation of this type has been reported.

Acid buffers. The use of acid buffers for 1–2 hr at 37°C at pH 3·0 and 3·2 (Lerner *et al.*, 1967) has been widely accepted for eluting antibodies from kidney. McPhaul & Dixon (1970) reported recovery of 60% of labelled protein from kidneys of monkeys injected with radiolabelled acid-eluted antibodies from human kidneys containing anti-GBM antibodies.

In the current investigation only 12–25% of sheep anti-GBM antibodies was eluted under similar conditions. The low recovery is probably due to the high avidity of the antibody (cf. results with differing

anti-BSA antibodies). Higher yields could be obtained with lower pH but at 37°C the ABC of the antibody became impaired. At 4°C, however, almost equal yields were obtained (a result consistent with the findings of Singer, Fothergill & Shainoff, 1960) and both anti-GBM antibodies and antibodies to BSA showed better preservation of function. Successive elution with acid buffers of high or low molarity at pH 3·2, 3·0, 2·8 and 2·6 for periods of 60 min at 4°C allowed the recovery of functionally active antibodies of progressively greater binding capacity both with anti-GBM antibodies and antibodies to BSA.

Elution of labelled BSA from kidneys of rabbits with chronic BSA serum sickness was achieved by Wilson & Dixon (1970) using buffers of pH 3·2. The yields obtained in the current experiments were low (25%) even with buffers of lower pH. This may be due to physicochemical changes occurring with BSA in acid solutions (Tandford *et al.*, 1955) or to erratic aggregation dependent upon a thiol-disulphide exchange (Bro, Singer & Sturtevant, 1958). On incubating ^{125}I-labelled BSA with kidney homogenates in acid buffers (0·02, 0·1 and 0·5 M and pH 2·6–3·2) up to 50% remained in the centrifuged sediment. The basement membrane swells but does not dissolve in dilute acid (Kefalides, 1969).

Alkaline buffers. In alkaline conditions both labelled BSA and anti-BSA were eluted rapidly and the functional integrity of antibodies was preserved between a pH range of 10·5–11·1 if procedures were carried out at 4°C. It is possible that elution under these conditions involved dissolution of immune aggregates. Kleindschmidt & Boyer (1952) found that egg albumin–anti-egg albumin precipitates were solubilized at pH 11. Wood *et al.* (1968) found that viable acid-sensitive virus could be desorbed from specific immunoadsorbents at pH 11·1 and Childhow, Bourne & Bailey (1974) reported successful desorption of specific collagen peptides using 1 M ammonia. Alkaline pH may solubilize membrane systems (Penefsky & Tzagoloff, 1971) and this may contribute to elution.

Chaotropic ions. Chaotropic ions have been reported to solubilize and dissociate immune precipitates (Dandliker *et al.*, 1967), dissolve glomerular basement membranes (Marquardt *et al.*, 1973) and C3-receptor complexes from lymphoid cells (Dierich & Reisfeld, 1975). They effectively eluted immune complexes, the yields reaching a maximum within 30 min. Potassium bromide at pH 9 gave a higher yield than sodium thiocyanate at pH 6·5.

A moderate to marked reduction in the ABC of anti-BSA antibodies was observed when these were incubated with chaotropes for 2 hr at 37°C in the presence of homogenized kidney; this effect was more pronounced with thiocyanate than bromide but did not occur when experiments were carried out at 4°C. Destructive effects of chaotropic ions may be partly mediated through their activity in promoting lipid auto-oxidation (Hatefi & Hanstein, 1970). This effect is more pronounced with thiocyanate than bromide and is favoured by elevated temperature and pH below 6·5. From the results we conclude that in order to dissociate immune complexes from kidney homogenates the best approach is to use a weak chaotrope buffered at pH 9, in two or three extractive steps of 30 min each, using ten parts of solution per one part of tissue at 4°C.

Kidneys from rabbits injected with radiolabelled anti-GBM antibodies were used to study the effects of chaotropes and the influence of different factors already discussed. The activity of anti-GBM antibodies eluted under these conditions could not be assessed because of concommitant extraction of large amounts of GBM antigens (Marquardt *et al.*, 1973).

Other eluting agents. 8 M urea at 4°C was capable of eluting more than 50% of antibody in the BSA system but was less effective than chaotropes in eluting antigen. Neither 2·5 M magnesium chloride nor 2 M sodium chloride was effective in eluting antigen or antibody in the BSA system at 37°C for 30 min. However, these agents allowed functional activity of antibody to be retained after 2 hr at 37°C and they may prove to be of particular value in studying immune complexes involving non-protein antigens, such as has already been reported by Koffler *et al.* (1971) for anti-DNA antibodies. The remaining agents tried (distilled water and 0·1 M EDTA) were ineffective and sodium dodecyl sulphate proved impracticable because of the extensive dialysis required to remove it.

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