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Physicochemical Characterization and Isolation of Rabbit Kidney-specific Autoantigens

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Summary. Rabbit kidney tissue contains antigens which are tissue-specific and species restricted, as well as other antigens which are shared by different organs of the animal. The stability of these antigens was investigated as a function of temperature, in order to explore the possibility of thermal fractionation. It was observed that one rabbit kidney-specific autoantigen was destroyed at 56° and another at 65°. A third antigen, which is restricted to the rabbit species but is non-tissue specific, was destroyed at 72°. Ultracentrifugal analysis of the saline extract at different concentrations showed the presence of several components, whose extrapolated values at zero concentration were found to be 4·2S, 6·2S, 10S, 19S, and 80S. The first two components were the most prominent.

The rabbit kidney-specific autoantigens were fractionated by a first step of salting out with ammonium sulphate at 4°. The fraction that precipitated in the range from 2·00 to 3·00 M retained most of the antigenic activity. As this fraction was quite impure, it was chromatographed through DEAE-cellulose, then processed through gel filtration columns, using Sephadex G-100 and G-200, and finally purified in agar zone electrophoresis. Two antigens were isolated and both shared similar antigenic characteristics. However, they had slight differences regarding their physicochemical properties. Sedimentation coefficients of 4·6S and 4·8S were obtained for them. Both antigens possessed a slow electrophoretic mobility, similar to that of the β-globulins. The antigen with higher sedimentation value was slightly faster electrophoretically than the other.

INTRODUCTION

Previous reports of our studies have described the production of autoantibodies in rabbits that had been isoimmunized with emulsions of Freund's adjuvant and kidney tissue (Centeno, Shulman and Milgrom, 1964; Milgrom, Centeno, Shulman and Witebsky, 1964; Centeno, Shulman, Milgrom, Witebsky and Skelton, 1966). It has also been shown that kidney tissue contains antigens which are tissue-specific and species-restricted, as well as some other antigens which are shared by different organs in the same animal (Centeno, Shulman, Milgrom and Witebsky, 1965). The present report describes some physical and chemical characteristics of saline extract of rabbit kidney and the isolation of the most active kidney-specific autoantigens.

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MATERIALS AND METHODS

Preparation of extract

Rabbit kidneys were supplied by Pel-Freez Biologicals, Rogers, Arkansas, and kept in the frozen condition until used. To prepare extracts, the organs were deprived of connective tissue and sliced before thawing, then weighed and ground with cold saline (0·15 M NaCl) in an Omni-mixer. Generally, the volume of liquid used was twice the weight of the tissue. To prevent any possible denaturation by excessive heating, the Omni-mixer chamber was always dipped in a mixture of ice, water, and salt to lower the initial temperature to $-8°$.

The homogenization was performed twice for 2 minutes each, with an interval to allow the mixture to cool. The suspension was centrifuged at 16,000 rev/min in a refrigerated machine, giving a red and turbid supernatant, and the sediment was processed as before, for three additional times. After this procedure, the sediment was resuspended in 4·0 M NaCl and processed in the Omni-mixer again, as described above. This was done to ensure a more complete extraction of the tissue components. Because the mixture was thick, the addition of distilled water was made for convenience, and the concentration of salt was decreased to 0·40 M; then the suspension was centrifuged again at 16,000 rev/min. Finally all supernatants were pooled and the insoluble debris discarded. For the determination of protein, the biuret method of Gornall, Bardawill and David (1949) was used; the extinction coefficient for this purpose was assumed to be 2·8, a value that had been obtained for a pooled serum preparation.

For the u.v. absorption at 280 mμ, an extinction coefficient was determined. For this purpose, a saline extract made of kidney tissue was thoroughly dialysed against 0·15 M NaCl, and samples of it were taken for dry weight at 105°, correcting for the salt content by dry weight determination on the final dialysis bath; this was accompanied by readings of optical density. The resulting value was 10·3.

Procedures for fractionation

Four methods have been used in the isolation of this rabbit kidney autoantigen, namely, salting out, chromatography on DEAE-cellulose, gel filtration using Sephadex, and agar zone electrophoresis.

(1) *Salting out.* The salting out was performed at 4° by drop-wise addition of the proper volume of 3·60 M ammonium sulphate. Three ranges of ammonium sulphate concentration were used, namely, from 0·00 to 1·40 M, from 1·40 to 2·00 M, and from 2·00 to 3·00 M. Each precipitate was removed by centrifugation, washed several times, redissolved in saline, and dialysed against this solvent. Suitable volumes of the stock solution of ammonium sulphate were then added to each supernatant to attain the desired salt level.

(2) *Chromatography.* DEAE-cellulose was obtained from Brown Company, Berlin, N.H., and was Type 20, reagent grade, capacity 0·93 mEq/g. The ion exchanger was washed in 0·5 N NaOH and then in distilled water. The alkaline treatment and the washing was repeated until the cellulose was quite white and the wash liquid colourless.

Buffers used in this fractionation are shown in Fig. 2. The resin was resuspended in the starting buffer, pH 7·8, and 0·02 M phosphate.

Columns of 2·5 cm internal diameter were used, pouring and packing the resin to a height of 60 cm. The columns generally had a flow rate of 5–8 ml/30 minutes. The starting buffer was then allowed to run through the column until the resin was equilibrated.

Fractions were collected by stepwise elution, using a GME time-activated fraction collector. Buffers with decreasing pH and increasing ionic strength were used. Optical density readings were taken with a Beckman DU spectrophotometer at 280 mμ, and every tube was examined. Buffers were changed after the optical density showed a steady and reasonably low value. Various fractions were pooled according to the outline of elution peaks, recorded by graphing the optical density versus the tube number. Pools were concentrated by the osmotic effects of dry sucrose.

(3) *Gel filtration.* Sephadex G-100 and G-200 were processed according to the specifications of Pharmacia Fine Chemicals Inc., and used in columns of 1 cm internal diameter and 130 cm height. As eluant, a solution of 0·60 M NaCl, buffered with phosphate, pH 7·4, 0·02 M was used. Fractions were collected and examined as described for chromatography.

(4) *Agar zone electrophoresis.* A 3 per cent solution of Difco Noble agar, dissolved in barbital buffer, pH 8·6, ionic strength 0·05, was poured in a plate 30 cm long, 5 cm wide, and 1 cm deep. At both ends of the plate two wicks of filter paper were placed as a bridge with the buffer baths, which contained the electrodes. Once the agar became solid, a trough 2 mm wide and 4·0 cm long was cut in the middle of the plate, perpendicular to the main axis.

Prior to the application of the sample, the plate was placed in the cold room, and a potential difference of 300 V was applied for a period of 1 hour. After this period, the sample was introduced inside the trough, and the power supply was connected again at the same voltage. After approximately 3 hours, as the trough became dry, it was filled with 3 per cent agar to reduce the possibility of breakage. After 24 hours the run was stopped; a thin slice was cut lengthwise and stained with bromophenol blue, thereby making it possible to locate the position of the main components. The plate was cut in transverse pieces 2 cm wide, starting from the cathode. Each piece of agar received a number and was then crushed and extracted with 10 ml of saline. These mixtures were kept for 24 hours at 4°, and were then centrifuged at 16,000 rev/min for 30 minutes, the supernatants were removed, and new portions of saline were added and processed a second time. Optical density at 280 mμ was taken for all the samples and the volumes recorded; the samples were pooled according to their positions on the elution graph. Each sample was concentrated and its antigenic activity as well as its homogeneity studied.

The preparative procedure is summarized in part of the flow sheet that is presented in the next paper (Shulman and Centeno, 1972).

Analytical methods

The different fractions, obtained either from salting out, chromatography, gel filtration, or agar zone electrophoresis, were tested for heterogeneity by means of three criteria: gel diffusion precipitation with appropriate antisera, filter paper electrophoresis, and analytical ultracentrifugation.

(1) *Gel diffusion and antisera.* The gel diffusion procedures have been described previously, as were also the characteristics of the anti-kidney antisera (Milgrom *et al.*, 1964; Centeno *et al.*, 1966). The antisera employed in the present investigation were R 1705 and R 1786.

(2) *Ultracentrifugal studies.* Ultracentrifugal studies were performed in a Spinco Model E instrument utilizing schlieren optics. A single-sector cell was used at the top speed of 60,000 rev/min, thus minimizing diffusion and therefore the problems of resolution. From a plot of the logarithm of the distance from the axis of rotation to the boundary (x)

against the time (t), the sedimentation coefficient was calculated. The RTIC unit provided the values of temperature as it changed during the run. The coefficients were corrected to the values that would be obtained at 20° in water, s_{20w}, recorded in Svedberg units and extrapolated to zero concentration. This extrapolation was done only with the rabbit kidney saline extract. A value of 0·75 was assumed for the partial specific volume.

(3) *Paper electrophoresis.* This type of electrophoresis was performed by the filter paper strip method, procedure B, as described by the manufacturer, using the Spinco Model R System.

RESULTS

PHYSICOCHEMICAL STUDIES

Some preliminary physicochemical studies were performed with the total saline extract of rabbit kidney. One study was concerned with the stability of the antigen as a function of temperature, to explore the possibility of thermal fractionation. The saline extract, at 2 per cent, was divided into several tubes containing 2·0 ml each and incubated for 30 minutes at several temperatures, namely, 37, 45, 56, 65, 72, and 85°. All tubes were subsequently centrifuged at 7000 rev/min for 20 minutes, and a gel diffusion test was set up on each supernatant, using antiserum R 1705. Of the three antigens that are indicated for the unheated extract, it was observed that one antigen was destroyed at 56° and another at 65°. Both of these antigens, according to studies which have already been published, are kidney-specific and have the properties of eliciting autoantibodies. There is a third precipitin band that was still seen after heating at 65°, but did not show up in the extract heated at 72°. This line is very faint in front of well 4, although it is a bit stronger (as the fainter and faster of the two lines) for well 3. As previously reported, this corresponds to an antigen restricted to the rabbit species but not tissue-specific (Centeno *et al.*, 1966).

Ultracentrifuge runs at different concentrations were also performed with rabbit kidney saline extract, and it was observed that there was a main component which had a sedimentation coefficient, at 1·0 per cent protein concentration, of 4·0S. Among the several minor components, the most prominent had a rate of 6·0S in this same run. When extrapolated values were obtained, these were found to be 4·2S and 6·2S respectively. Several quite minor peaks were estimated to have rates of 10S, 19S, and 80S.

Paper electrophoresis studies indicated that there was a main component in the kidney extract whose mobility was similar to that of serum albumin, and that there were components with mobilities ranging from α- to γ-globulins, but it was not possible to see a clear resolution between them. This pattern will be illustrated at a later point, in comparison to some fractionated products (Fig. 1).

FRACTIONATION STUDIES

After fractionation by precipitation with ammonium sulphate and dialysis against saline, the various fractions, all at the same protein concentration, were tested for antigenic activity, using gel diffusion plates. With all the samples having essentially the same total concentration, it was possible to observe that the fraction which precipitated in the range from 2·00 to 3·00 ammonium sulphate had the strongest precipitating activity of the type associated with the kidney-specific antigen; it always showed the sharpest immunoprecipitin band. The comparative degrees of physicochemical heterogeneity of these three

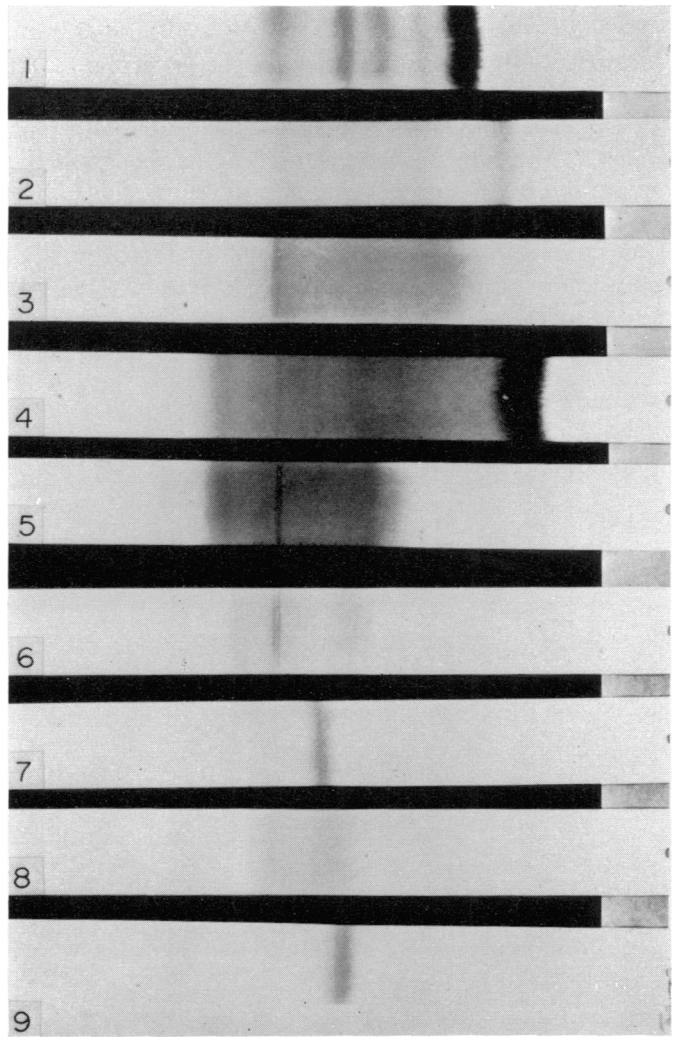

FIG. 1. Filter paper electrophoresis patterns. (1) Normal rabbit serum; (2) rabbit kidney saline extract; (3) fraction precipitated with ammonium sulphate at 1·40–2·00 M; (4) fraction precipitated with ammonium sulphate at 2·00–3·00 M; (5) fraction eluted at pH 7·8 from a DEAE-cellulose column; (6) sample applied to an agar zone electrophoresis experiment; (7) fraction 5 from agar zone electrophoresis; (8) fraction 7; and (9) fraction 9, both from the above-mentioned agar zone electrophoresis experiment.

fractions was studied by filter paper electrophoresis, as shown in Fig. 1, along with comparisons to the whole saline extract (as mentioned above) and to rabbit serum. Our efforts were subsequently directed to the fraction, 2·00–3·00 M, which, regardless of its polydispersity, as revealed by immunochemical, electrophoretic and ultracentrifugal analysis, proved to have the highest relative concentration of the kidney-specific autoantigen, as compared with the other fractions obtained by salting out with ammonium sulphate. Reprecipitation with ammonium sulphate gave a fraction which looked more highly purified, according to gel diffusion and paper electrophoresis, but it was by no

means a homogeneous material. Thus, the fraction that had been originally precipitated in the range of 2·00–3·00 M was applied to a DEAE-cellulose column and eluted with different buffers. A representative elution pattern is shown in Fig. 2, along with the composition of the various buffers used in this fractionation. The fraction eluted with buffer of pH 7·8 showed activity; in all other fractions, no antigen was detected. This first fraction constituted 51 per cent of the total material eluted. This was confirmed by several such chromatographic experiments. Every tube with suitable optical density readings were checked for antigenic activity in gel diffusion plates; otherwise, several of them were concentrated and then tested.

Since a significant amount of antigen came out with buffer of pH 7·8, a pool was made with all the fractions eluted by this buffer, the volume was reduced, and studies were performed to determine the degree of heterogeneity. Since this preparation did not provc

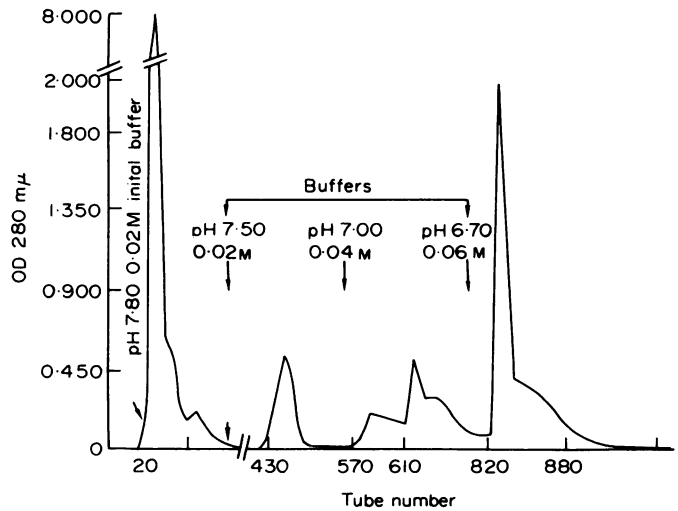

FIG. 2. Chromatography elution from DEAE-cellulose of a rabbit kidney fraction obtained by precipitation with ammonium sulphate in the range of 2·00–3·00 M. The sample was 454 mg of protein in a volume of 7 ml. Flow rate was 6 ml per 30 minutes. The small arrows indicate the location of the antigenic activity.

to be highly homogeneous, as shown quite well in strip 5 of Fig. 1, further manipulations were necessary. Consequently, the pool was applied to a Sephadex G-100 column. The elution pattern showed a large asymmetric peak with a shoulder. This is shown in Fig. 3. Testing the tubes individually for precipitating activity, positive reactions were obtained with tube 6 (first arrow), and this reactivity increased to a maximum in tube 7 (second arrow); beyond this point the activity decreased, and in tube 9, even though the protein concentration reached its highest value, there was no activity at all, as shown in gel diffusion. The tubes having antigenic activity were pooled again and applied to a column of Sephadex G-200. The elution pattern, as shown in Fig. 4 had three peaks, and the activity, following the procedure described before, was restricted to the samples obtained in a small range of fractions, as indicated by the arrows in Fig. 4. A pool was then made with the tubes having the highest activity within this range. This pool was rerun in Sephadex G-200, and the resultant elution pattern was found to show only one fraction. This pattern is seen in Fig. 5.

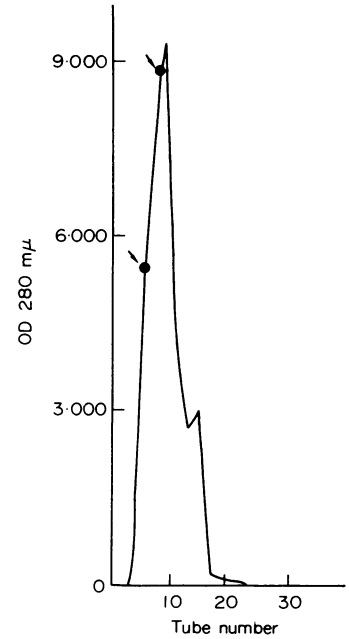

FIG. 3. Elution pattern from a Sephadex G-100 column of a fraction eluted at pH 7·8 from DEAE-cellulose. The sample was 227 mg in a volume of 5 ml. The arrows indicate the location of the antigenic activity.

The antigenic activity increased and decreased with the protein concentration and was observed both at the leading and trailing edges of the peak. The samples with activity (tubes 58–71) were pooled. When the sample was studied in paper electrophoresis,

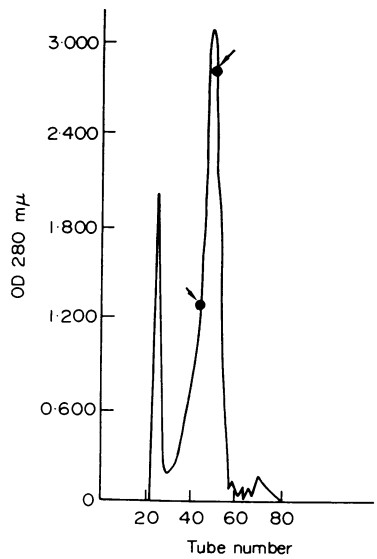

FIG. 4. Elution pattern from a Sephadex G-200 column of the active fraction obtained from Sephadex G-100. The sample was 75 mg in a volume of 2 ml. The arrows indicate the location of the antigenic activity.

although exhibiting an improved homogeneity, it still had three bands (strip 6 in Fig. 1). Therefore, a last preparative step was performed, using agar electrophoresis, whose elution profile is shown in Fig. 6.

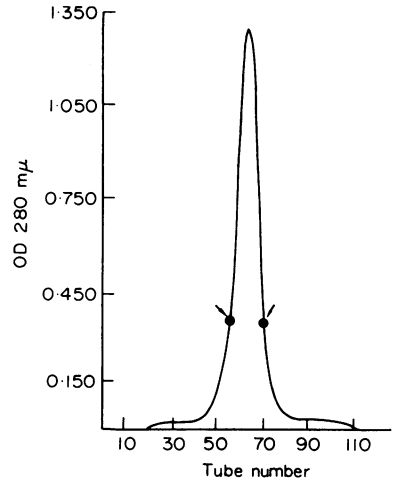

FIG. 5. Elution pattern of the active fraction obtained from a Sephadex G-200 column, re-filtered on the same column. The sample was 12 mg in a volume of 1 ml. The arrows indicate the location of the antigenic activity.

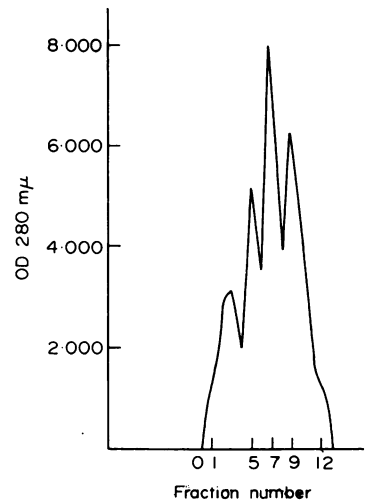

FIG. 6. Elution diagram from agar zone electrophoresis. The sample was 54 mg.

The first elution liquid coming from the different segments was tested in gel diffusion without any other treatment. Under these conditions, it was observed that the fractions from segments 5, 7, and 9 had activity. These fractions were concentrated and it was observed that the fraction 7, corresponding to the application point, showed a broad

precipitin band which merged with those developed by the other fractions (Fig. 7). These fractions were also studied by means of paper electrophoresis and ultracentrifugal analysis. Paper electrophoresis (Fig. 1) showed for either fractions 5 or 9, a single band of different mobilities, approximately that of a β-globulin, being slightly faster for fraction 9. It should be understood that only the band on the right is under consideration in these three strips; the left-hand band is mostly residual material at the point of origin. With regard to fraction 7, there were two bands, and each of them was related to the component in 5 or in 9. When ultracentrifugal analyses were made, the results correlated

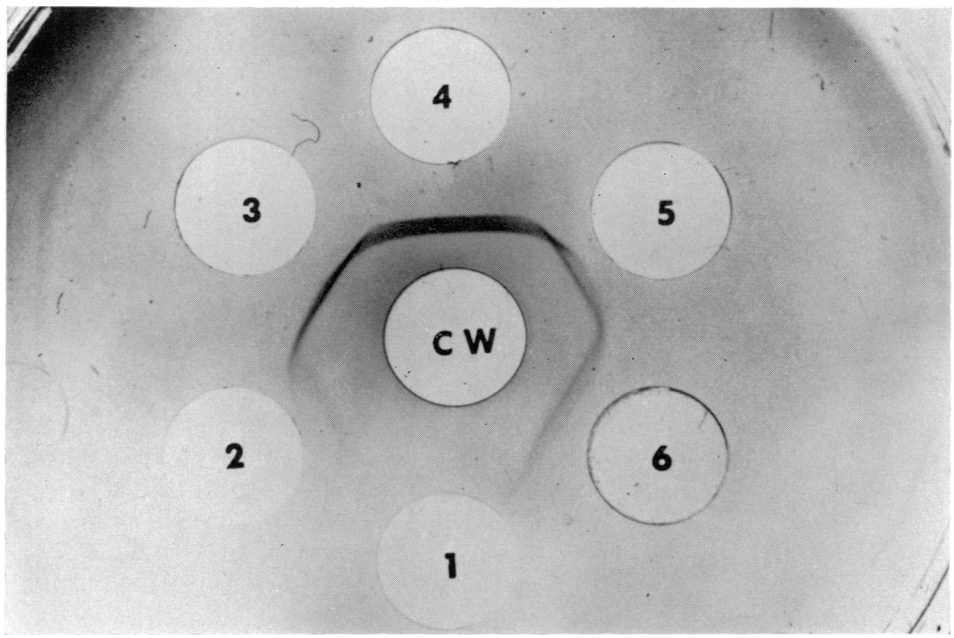

FIG. 7. Gel diffusion precipitation pattern made with the various fractions. Central well: rabbit serum R 1705. Peripheral wells: (1) fraction 2, concentration 0·38 per cent; (2) fraction 3, concentration 0·38 per cent; (3) fraction 5, concentration 0·60 per cent; (4) fraction 7, concentration 0·96 per cent; (5) fraction 9, concentration 0·70 per cent; (6) fraction 10, concentration 0·50 per cent.

well with the findings just described. While fractions 5 and 9 showed a single boundary with a sedimentation coefficient of 4·60S and 4·80S respectively, the fraction 7 showed both components, as shown in Fig. 8.

The immunological relationship between the fraction 5 obtained from zone electrophoresis in agar and rabbit kidney total saline extract is shown in Fig. 9. By this criterion also, this final product, fraction 5, is seen to be quite homogeneous and strongly reactive with its antibody. In this particular plate, serum rabbit R 1786 was used. The second precipitin band observed in the picture corresponds to an antigen shared by several organs in the rabbit. The immunological characteristics of these antigens have previously been described (Centeno *et al.*, 1966). The isolation of this antigen is the subject of a following paper (Shulman and Centeno, 1973).

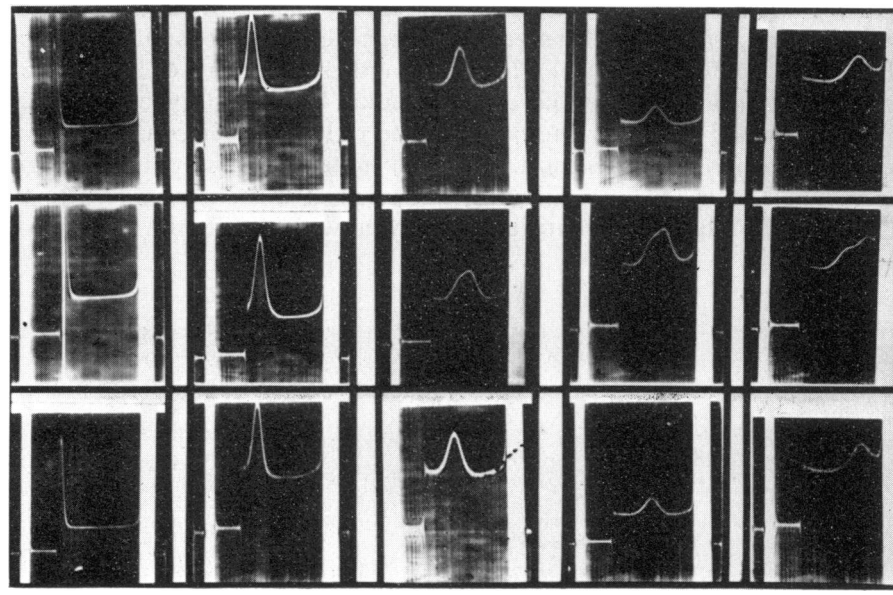

FIG. 8. Ultracentrifugal patterns of fractions 5, 7, and 9 (in upper, middle, and lower rows, respectively) from agar zone electrophoresis. Protein concentrations: 0·60, 0·96 and 0·70 per cent, respectively. Picture taken at 4, 24, 32, 40, and 68 minutes after the rotor reached the speed of $59{\cdot}8 \times 10^3$ rev/min. Direction of sedimentation is to the right.

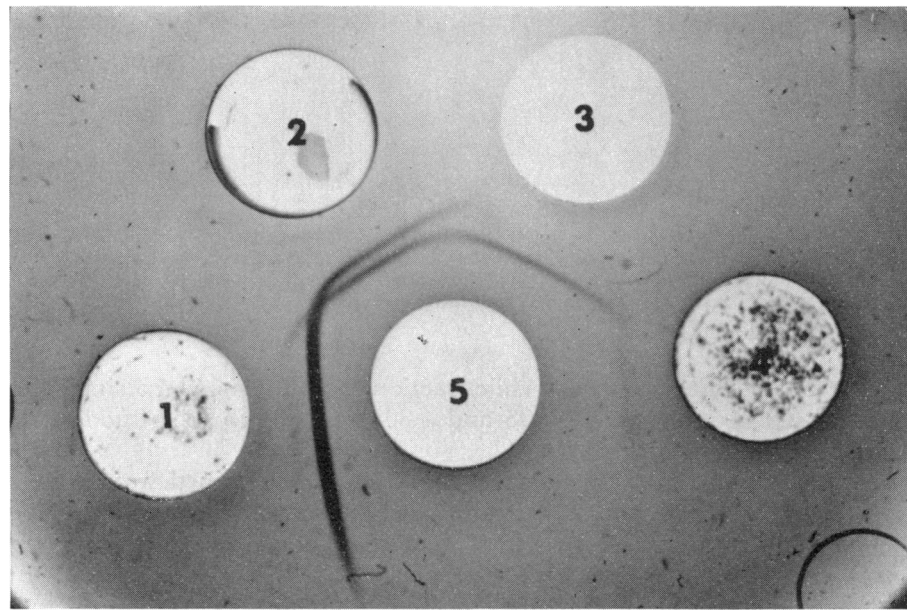

FIG. 9. Gel diffusion precipitation pattern. Central well: serum from R 1786. Peripheral wells: (1) rabbit kidney specific antigen fraction 5, concentration 1·50 per cent; (2) rabbit kidney saline extract, concentration 3·0 per cent; (3) rabbit kidney non-specific antigen, concentration 1·2 per cent; (4) fraction not related to the present study.

DISCUSSION

Rabbit kidney tissue extracts are very heterogeneous, as demonstrated by physicochemical procedures which revealed several macromolecular components, most likely proteins. Members of this conglomerate of substances are the antigens whose immunological characteristics were described previously (Centeno *et al.*, 1966). Thus, ultracentrifugal studies showed several components whose sedimentation coefficients, extrapolated to zero concentration, were: 4·2S for the main one, 6·2S for the constituent in second greatest quantity, and finally 10S, 19S, and 80S for some others in very low concentration.

Analyses performed in paper electrophoresis gave results that agreed with the abovementioned observations and showed a main component with the mobility of albumin, and a group of polydisperse substances spreading all over the electrophoretic field from the γ-globulin region to that of the albumins, without any interruption between component and component.

The effect of the temperature on the antigenic properties of these substances was studied. It was observed when antiserum R 1705 was used that the kidney-specific antigen is denaturated between 45° and 56°. The second kidney-specific autoantigen was destroyed at 65° when it was heated for a term of 30 minutes. These details supported the idea of a protein nature for these antigens.

From a system as complex as this is, two autoantigenic substances have been isolated in a high state of purity (fractions 5 and 9), considering as criteria of purity the following parameters: (1) a single band in paper electrophoresis; (2) a unique component according to analytical ultracentrifuge; and (3) a single precipitin band on agar immunodiffusion. Both isolated antigens have very slight differences in their physicochemical properties; however, they share similar antigenic characteristics.

The use of DEAE-cellulose chromatographic columns have provided a gentle method for removing contaminants without altering the properties of the antigens, since they were not absorbed to the resin, but passed through it; moreover, this was a quite helpful process of purification, since it permitted us to work with relatively large quantities of material in a short period of time, without the necessity of using several buffers, with the pertinent delays, until the different fractions were eluted from the columns. Though the process itself did not produce a noticeable amount of denatured protein, the unavoidable handling of the fractions, dilutions, and concentrations caused losses in activity or manifest denaturation that were shown as precipitates. The instability of these antigens was an important factor among the difficulties in isolating them. So, it is clear that the quantities recovered in each step have decreased notably, regardless of all precautions taken, including low temperature.

Sephadex G-100 and G-200 were also used. The simple observation of the elution diagram shows the proportions of the antigens contained in the material chromatographed. However, it was finally possible to obtain a fraction antigenically homogeneous although physically heterogeneous, whose components were isolated in a last step by means of zone electrophoresis. Both isolated antigens have sedimentation coefficients with very similar values.

Even though there are differences in mobilities, either by ultracentrifugal or electrophoretic methods, there are no differences from the point of view of their antigenicity, and apparently both fractions 5 and 9 shared the same antigenic determinants. There is one point that strongly supports this postulation, and this is the fact that the concentration of

fraction 7 is less than the other two combined; therefore, if the proportions of 5 and 9 were equal, the antigenic activity of the fraction 7 should be weaker than either of the other two components, but this fact does not happen, and fraction 7 behaves as only one antigenic component, despite its demonstrated heterogeneity.

The antigenic relationship between these two antigens is interesting, as it could also happen that they were not really two molecular entities detectable in a single individual, but variations in a protein structure. It is easy to realize that there is a complexity of the genetic mosaic for the constituents in the initial extract. Partial denaturation may have happened to this antigen, because of the different processes to which it was subjected. The immunological identity between the isolated material and the antigen contained in the

TABLE 1

YIELDS OBTAINED IN A SEQUENCE OF EXPERIMENTS STARTING WITH THE PREPARATION OF RABBIT KIDNEY SALINE EXTRACT AND ENDING WITH THE PRODUCT WHICH FINALLY WILL BE PURIFIED BY ZONE ELECTROPHORESIS

Rabbit kidney homogenized	361 g
Protein extracted	18
Fractionation with ammonium sulphate	
Protein recovered from the material precipitated in the range 0·00–1·40 M	576 mg
Protein recovered from the material precipitated in the range 1·40–2·00 M	801
Protein recovered from the material precipitated in the range 2·00–3·00 M	566
Fractionation by chromatographic means	
Sample (from 2·00–3·00 M) applied onto a DEAE-cellulose chromatographic column	454
Active fraction obtained from this column	245
Sample chromatographed in Sephadex G-100	227
Active fraction recovered from this column	75
Sample chromatographed in Sephadex G-200	75
Active fraction recovered from this column	12
Sample re-chromatographed in Sephadex G-200	12
Active fraction recovered from this column	8
Percentage of active substance recovered from 361 g of rabbit kidneys	0·003

initial extract, however, is demonstrated by the merging of the precipitin bands formed between the antibodies contained in the antiserum and the above-mentioned antigens. The yields, Tables 1 and 2, show clearly the very small amount of material recovered.

It may be emphasized that the isolated antigens are not only tissue-specific, but also species-specific, as was shown in previous papers (Milgrom *et al.*, 1964; Centeno *et al.*, 1966).

Rabbits immunized with homologous kidney saline extract developed mild lesions in the kidneys characterized by proteinuria, cellular proliferation, and massive tubular calcification (Centeno *et al.*, 1966; Centeno and Shulman, unpublished). The capacity of homologous kidney to elicit autoantibodies in rabbits has been confirmed in several important publications (Unanue, Dixon and Feldman, 1967; Lerner and Dixon, 1968; Edgington, Glassock and Dixon, 1968); however, there is not, as yet, a clear knowledge of the relationship between the already-recognized antigens and the ones dealt with in this paper. This is a problem that can only be elucidated by isolating each antigen and studying the individual immunological responses.

TABLE 2

RECOVERY OBTAINED FROM ZONE ELECTROPHORESIS USING AGAR AS SUPPORTING MEDIUM

Sample applied into the agar, expressed as protein Protein recovered from the different fractions:		Per cent active substance 54 mg
Fraction number	Protein mg	
1	—	—
2	3·4	—
3	3·4	—
4	2·5	—
5	6·0	11
6	3·8	—
7	9·6	18
8	3·7	—
9	7·0	13
10	5·0	—
11 and 12	1·1	—
Total Protein recovered per cent: 84·2	45·5	

The exact role of the various kidney antigens, i.e. of the specific and non-specific antigens of the tubules and of the glomerulus, in the pathogenesis of kidney lesions is difficult to assess. The isolation of some of these antigens may enable one to delineate in a clearer manner the function played by each in the development of the damage. Additional studies have recently been reported on the group of kidney-specific antigens that can be isolated from rabbit kidney (Shulman and Wypych, 1969). The subject of kidney antigens and of the various forms of kidney autoimmunity have been extensively reviewed elsewhere (Shulman, 1973).

It is difficult to make any assumption on the role that the particular antigen described in this paper could play in the induction of lesions in rabbit kidney; however, the fact that it can be obtained in a very high state of purity provides a perspective for studying the immunologic response to be obtained in rabbits immunized with it, and to study further its pathological consequences to the tissues.

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