Solubilization and Some Characterization of Chicken Kidney Microsomal Antigens

By

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ABSTRACT "Kidney-specific" antigens are contained in the subfraction salted out of the deoxycholate-soluble fraction of chicken kidney microsomes between 25% (or 15%) and 45% saturation of ammonium sulfate. This subfraction is characteristically rich in phospholipid-phosphorus. The same "kidney-specific" antigens can be isolated as a saline-soluble form without any change of the serological specificity. For this, the microsomes were treated with organic solvent and extracted with tris-saline. Such extract contains less than 5% of lipid contained in the deoxycholate-soluble fraction, but reveals "kidney-specificity" by immunological tests *in vitro.* Efforts for purifying "kidney-specific" antigens by means of Sephadex gel filtration and of density gradient centrifugation were described. "Kidney-specific" microsomal antigens of chicken were indetectable in the corresponding fractions of Japanese quail kidneys and of mouse kidneys.

Introduction

The initial step of histogenesis can be recognized as an initiation of the synthesis of the molecules which will specifically localize in each tissue. In order to detect these molecules, the immunological technique is promising for its high specificity and sensitivity.

Various tissue- or organ-specific antigens have been reported (5, 15, 16, 18, 20, 21, 35) and many efforts have been made to use them as a marker to trace differentiative changes of cells at subcellular level (7, 16, 22, 23, 29, 30, 36, 37). But little is known as to the chemical nature of such antigenic molecules.

In this paper, some characterizations of "kidney-specific" microsomal antigens of chicken will be reported together with the procedures to isolate them as soluble form. The interspecific distributions of the antigens among some Ver-
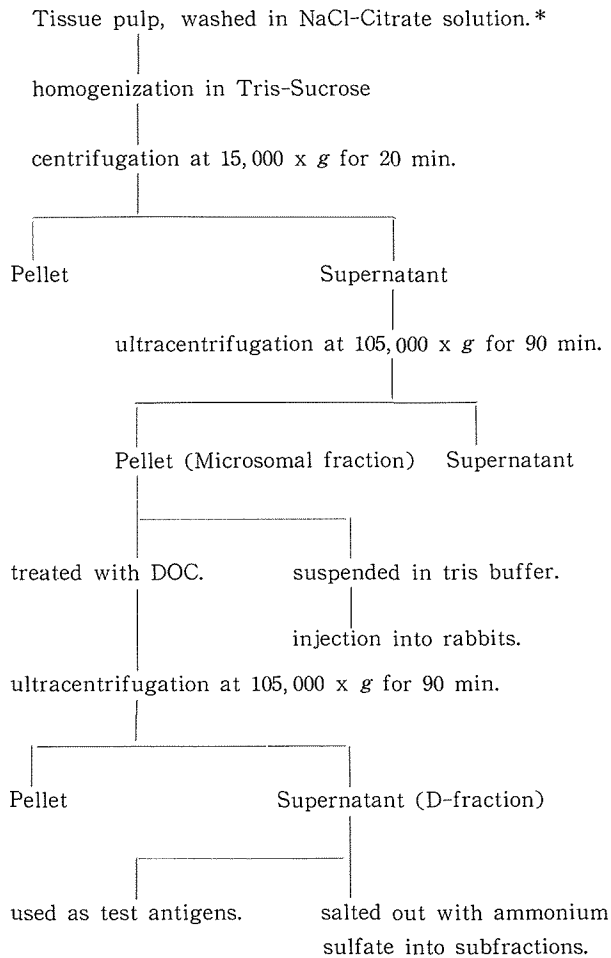
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tebrates animals are also discussed briefly.

Materials and Methods

Adult chicken kidney was mainly used as material.   Several other organs of chicken as well as kidneys of mouse and of Japanese quail, *Coturnix coturnix japonica*, were also employed in some tests. Freshly removed kidneys were rinsed in icecold citrated NaCl solution *, and were cleaned from the adherent connec-

Fig. 1.   Flow sheet of isolation of subcellular fractions.

Tissue pulp, washed in NaCl-Citrate solution. *
|
homogenization in Tris-Sucrose
|
centrifugation at 15,000 x g for 20 min.
|
Pellet   Supernatant
|
ultracentrifugation at 105,000 x g for 90 min.
|
Pellet (Microsomal fraction)   Supernatant
|
treated with DOC.   suspended in tris buffer.
|
injection into rabbits.
|
ultracentrifugation at 105,000 x g for 90 min.
|
Pellet   Supernatant (D-fraction)
|
used as test antigens.   salted out with ammonium
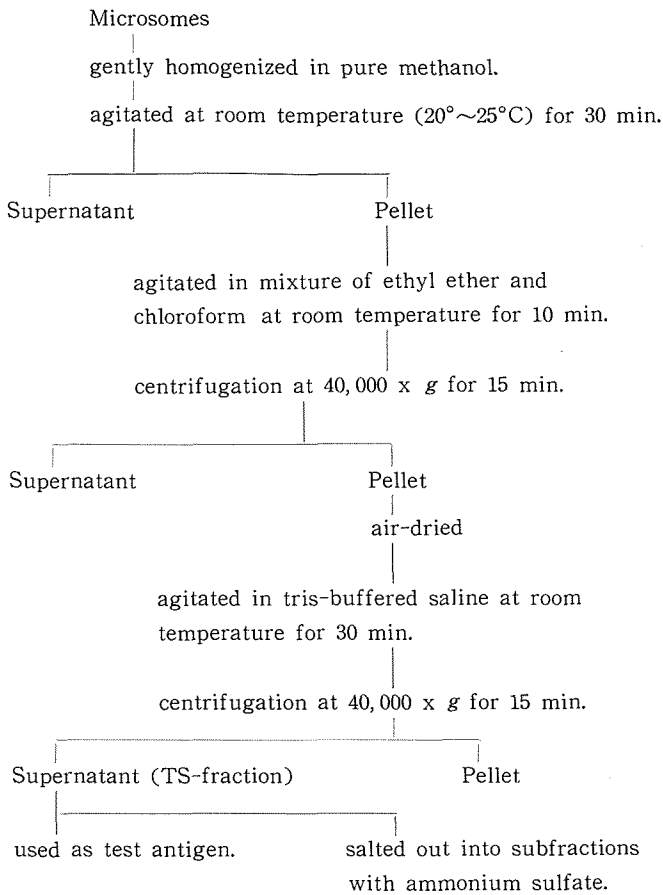   sulfate into subfractions.

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* NaCl-Citrate solution: Sodium chloride; 5.85g. Sodium citrate (Dihydrous tri-
      sodium salt); 29.4g. Distilled water; up to 1 liter.

tive tissues and blood as much as possible.   The washed kidney pulps were
stored at –20°C until analysis.

Preparation of Microsomal Fraction

The washed tissue pulps were homogenized in the icecold Tris–Sucrose
solution * (pH 7.8) with a Waring blendor for 3–5 minutes.   The homogenate
was spun at 13,000 x *g* for 20 minutes to remove cell debris, nuclei and mitochon-

Fig. 2.   Solubilization of the microsomal antigens without
                an use of detergent.

Microsomes
|
gently homogenized in pure methanol.
|
agitated at room temperature (20°~25°C) for 30 min.
|
┌───────────────────────────────────┐
Supernatant   Pellet
                                     |
             agitated in mixture of ethyl ether and
             chloroform at room temperature for 10 min.
             |
             centrifugation at 40,000 x *g* for 15 min.
             |
┌───────────────────────────────────┐
Supernatant   Pellet
                                     |
                                     air-dried
                                     |
             agitated in tris-buffered saline at room
             temperature for 30 min.
             |
             centrifugation at 40,000 x *g* for 15 min.
┌───────────────────────────────────┐
Supernatant (TS-fraction)   Pellet
|───────────────────────────────────┐
used as test antigen.   salted out into subfractions
                              with ammonium sulfate.

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* Tris-Sucrose solution :   Tris (Trishydroxymethylaminomethane);   6. 05g.
      Hydrochloric acid; up to pH 7.8.   Sucrose; 85.5g.   Magnesium chloride;
      1.0g.   Distilled water; up to 1 liter.

dria. The supernatant was spun at 105,000 x g for 90 minutes. The pellet thus obtained will be denoted as a microsomal fraction (3).

Solubilization of Microsomal Antigens

1) Solubilization with detergent. Microsomes were dispersed in tris buffer pH 7.8 *. An aqueous solution of sodium deoxycholate (DOC) was poured into the suspension up to the final concentration of 0.8%, and was spun at 105,000 x g for 90 minutes. The supernatant fluid thus obtained will be hereafter referred as D-fraction. The procedure is summarized as the flow sheet of Fig. 1.

2) Solubilization without detergent. Microsomes were washed in pure methanol at room temperature between 20° and 25°C. After centrifugation, the sediment was washed in ether-chloroform (2:1m) ixture. The washed microsomes were air-dried and stored at -20°C. The tris-buffered saline extract of this microsomal powder will be hereafter referred as TS-fraction (Fig. 2).

Table 1. Gross chemical composition of each subfraction.

Tissues	Preparations	Protein %	Ph-L-P %	Ph-L-P / Protein	RNA / Protein
Kidney	R			0.021	0.17
	D	100	100	0.036	0.047
	SF 0/10	17	5	0.004	0.040
	SF 10/15	9	10	0.036	0.032
	SF 15/50	40	39	0.040	0.018
	SF 50/70	10	2	0.0087	0.039
Liver	D	100	100	0.019	0.023
	SF 0/10	24	5	0.003	0.004
	SF 10/15	7	3	0.005	0.008
	SF 15/50	42	81	0.025	0.009
Lung	D	100	100	0.028	0.053
	SF 0/10	--	--	-----	-----
	SF 10/15	20	14	0.020	0.030
	SF 15/50	14	26	0.051	0.050

Ph-L-P: Phospholipid-phosphorus. R: DOC-insoluble fracticn of microsomes. D: D-fraction in the text. S: Postmicrosomal supernatant. SF 0/10: Subfraction from D-fraction precipitated between 0 and 10% saturation of ammonium sulfate. SF 10/15: Subfraction from D-fraction precipitated between 10 and 15% saturation of ammonium sulfate. SF 15/50: Subfraction from D-fraction precipitated between 15 and 50% saturation of ammonium sulfate.

* Tris buffer: Tris; 6.05g. Hydrochloric acid; up to pH 7.8. Distilled water; up to 1 liter.

Subfractionation with Salting Out Method

Salting out was undertaken by adding aqueous saturated ammonium sulfate solution gradually into D- or TS-fraction of the microsomes. Throughout the whole process, pH was maintained at 7.8 by adding a few drops of 2M-trishydroxy-methylaminomethane (Tris). The precipitate salted out with several different concentrations between 10 to 70% of saturations (Tab. 1) was dissolved in tris-buffered saline, and was dialyzed against the saline until ammonium ion became indetectable in the dialyzate by Nessler's reagent.

Preparation of Antisera

Fresh microsomes from the kidney homogenates were suspended in tris buffer, emulsified with an equal volume of Freund's complete adjuvant (obtained from Difco Laboratories), and were intraperitoneally injected into albino rabbits. Total doses of antigen reached 120mg-protein after 8 times' injections at intervals of six days. Two weeks after the final injection the animals were bled and the antisera were collected.

Crude globulin fraction was salted out from the antisera with ammonuim sulfate at 50% saturation, dissolved in tris-buffered saline, and was dialyzed against it.

Antiserum against the subfraction K45 which was salted out from D-fraction with the concentration of between 25% and 45% saturation of ammonium sulfate was prepared in the same way (anti-K45) (20). Each antiserum was decomplimented at 560°C for 30 minutes.

Immunological Tests in Vitro

Double diffusion method of Ouchterlony in agar gel (26) and ring precipitin test were applied. In double diffusion technique, the concentration of agar (Bactoagar obtained from Difco Laboratories) was 1% in 1/20M-tris-buffered saline at pH 7.8, containing 0.01% of sodium merthiolate.

In ring precipitin test serial dilutions of test antigen solutions were made with tris-buffered saline for simi-quantitative assay.

Chemical Analysis of Antigens

Each antigen sample was separated into protein, ribonucleic acid and phospholipid, according to Schmidt–Thannhauser's method (34). An amount of protein was determined by the phenol reagent of Folin–Ciocalteu (8), and that of ribonucleic acid, by the orcinol reaction against ribose (4). A relative amount of phospholipid was estimated upon phospholipid–phosphorus. For this, lipid fraction from each sample was digested in diluted perchloric acid (2). Inorganic phosphorus converted from phospholipid-phosphorus was thus obtained, and it was assayed by Allen's method (2). For a gross estimation of protein and ribonucleic acid in small samples, an ultraviolet absorption of the samples was often measured. The ratio of RNA/protein was calculated from the ratio of the optical density at $280m\mu$ to that at $260m\mu$.

Preparation of Polysaccharide from Kidney Microsomes

Polysaccharide was isolated from kidney microsomes using hot phenol method by Kabat and Mayer (10).

Sephadex Gel Filtration

Concentrated TS-fraction from kidney microsomes was applied in the Sephadex G-100 column with one centimeter in diameter and 60 centimeters in height. Elution was undertaken by 1/20M-tris-buffered saline.

Sucrose Density Gradient Ultracentrifugation

The concentrated T-SF11 subfraction, which is the subfraction salted out between 45% and 67% saturation of ammonium sulfate from TS-fraction, was spun in the sucrose gradient. The details of the conditions applied will be given later.

Results

Antigenic Constitution of D-fraction from Kidney Microsomes

In the reaction of D-fraction from kidney microsomes against anti-KMc (antiserum against chicken kidney microsomes) in agar gel, eight to ten precipitin lines are observed and classified into three groups (Fig. 3). Two or three lines,
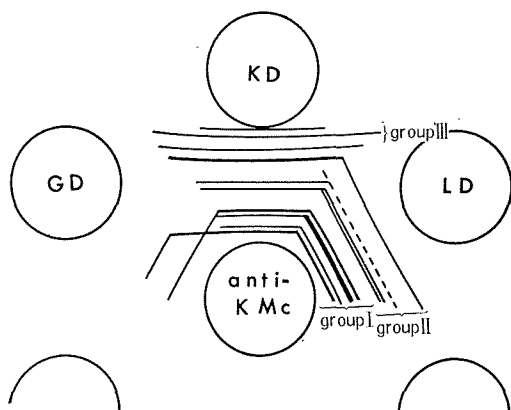
Fig. 3. Schematic diagram of precipitin lines.
KD: D-fraction of the kidney microsomes.
GD: D-fraction of the lung microsomes.
LD: D-fraction of the liver microsomes.
anti-KMc: antiserum against the kidney microsomes.

belonging to the group lll, are considered to be "kidney-specific". See the previous publication for the details in the reaction patterns (21).

The "kidney-specific" antigens are found in the subfraction salted out from D-fraction between 25% and 45% saturation of ammonium sulfate (SF25/45). Though this sample is far from purely "kidney-specific" and contaminated with non-specific antigens, injection of this subfraction into rabbits leads to the pro-

duction of relatively "kidney-specific" antiserum (anti-K45) in comparison with anti-KMc (20).

About 600 milligrams of proteins were yielded as D-fraction from 120 grams of fresh chicken kidneys. Results of a gross chemical analysis of this fraction and its subfractions are shown in Table 1. Subfraction SF15/50 from kidney microsomes, to which "kidney-specific" antigens are mainly distributed, is characteristically rich in phospholipid-phosphorus and relatively poor in ribonucleic acid. The similar properties are seen in the corresponding subfraction from both liver and lung microsomes.

Inhibition Test of Antiserum by Polysaccharide from Kidney Microsomes

An ability for polysaccharide from kidney microsomes to inhibit any antibodies contained in anti-KMc was examined. There was no difference in the pattern of the precipitin lines by Ouchterlony test between the polysaccharide-treated antiserum (250mg. polysaccharide per ml. of antiserum) and the non-treated antiserum.

Antigenic Constitution of TS-fraction

A treatment of the microsomes with methanol and subsequently with ether-chloroform resulted in remarkable decrease of viscosity of microsomal particulates. When tris-buffered saline extract from such treated kidney microsomes (TS-fraction) was tested against anti-K45 in agar gel, all of the antigens, found in D-fraction, were also detectable (Fig. 4).
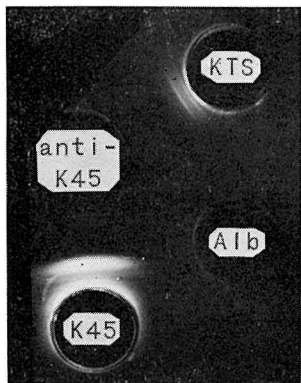
Fig. 4.   Serological reactivity of
TS-fraction.

anti-K45:   antiserum against SF25/45 from chicken
            kidney microsomes.
K45:   SF25/45 from chicken kidney microsomes.
KTS: TS-fraction from chicken kidney microsomes.
Alb: purified chicken serum albumin.

Each antigen solution contains 5 milligrams of protein
per milliliter. See the text for further details.

About 100 milligrams of protein were yielded as TS-fraction from 120 grams of the fresh kidneys. Contents of protein, phospholipid-phosphorus and ribonucleic acid in TS-fraction were measured and were compared with those in D-fraction (Tab. 2). More than 95% of phospholipid-phosphorus contained in D-fraction was removed in TS-fraction, whereas an increase in relative ribonucleic acid content was noticed. Enzymatic activity of TS-fraction from kidney microsomes

was preliminarily assayed. Activity of alkaline phosphatase (by Markert's me-
thod (14)) and that of esterase (by Allen's method (1)) were preserved in
TS-fraction with about two thirds' decrease in comparison with D-fraction in
kidney microsomes.

Table 2. Comparison of D- and TS-fraction.

	D-fraction	TS-fraction
Phospholipid-phosphorus μg/mg-protein	47	0.4
RNA μg/mg-protein	36	90
Precipitation with ammonium sulfate*	between 25% and 45%	between 45% and 67%

* The ranges in which the "kidney-specific" antigens are salted out by
ammonium sulfate are given.

Antigenic Costitution of the Subfractions Separated from TS-fraction

When salted out, TS-fraction from kidney microsomes yielded only a little
precipitate up to 45% saturation of ammonium sulfate. In the reaction against
anti-K45, the subfraction precipitated from TS-fraction between 45% and 67%
saturations of ammonium sulfate (T-SFII), contains all the antigens which are
identified as "kidney-specific". Contamination with non-specific ones is less
heavy here than in subfraction SF25/45 from D-fraction.

Separation of "Kidney-specific" Antigens from TS-fraction with Sephadex Gel Filtration

Figure 5 shows the optical density curve at 280mμ of the samples separated
from TS-fraction by Sephadex G-100 gel filtration. Two major peaks and a
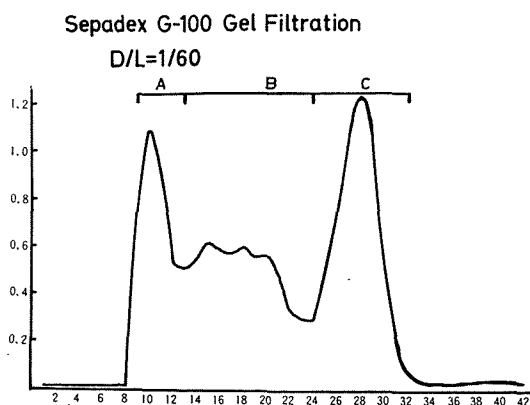
Fig. 5. Optical density curve of TS-fraction after Sepha-dex G-100 gel filtration.
Abscissa: tube numbers.
Ordinate: optical density at 280mμ.
Each tube contains about 2.5ml. aliquots.
See the text for further details.

plateau between them are designated as fraction A (tube numbers 8–13), C (tube numbers 25–30) and B (tube numbers 14–24) respectively. Each fraction, after being concentrated to an approximately equal protein concentration, was tested against anti–K45 in agar gel (Fig. 6). No precipitin line was observed with regard to fraction C, except one non–specific precipitin line observed closest to the antibody well. All other antigens contained in TS–fraction, irrespective of "kidney–specific" or non–specific, were detected only in fraction A in reaction with anti–K45.　Fraction B did not contain any test–antigens to react with anti–K45 under the present technique.
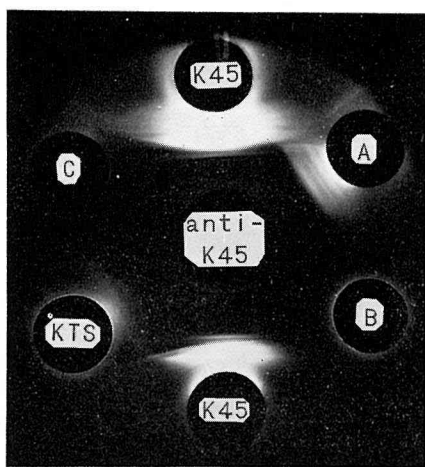
Fig. 6. Serological reactivity of the fractions separated by Sephadex gel filtration.

A: fraction A (tube numbers 8–13).
B: fraction B (tube numbers 14–24).
C: fraction C (tube numbers 25–30).

Each antigen solution contains 3 milligrams of protein per milliliter.
See Fig. 5 also.

Density Gradient Ultracentrifugation of TS-fraction

Concentrated T-SFII from kidney microsomes was spun in the sucrose gradient. In each run, optical density at $280m\mu$ and $260m\mu$ of each ten drop aliquot of the resulting sample was measured. Figure 7 shows the result of the run
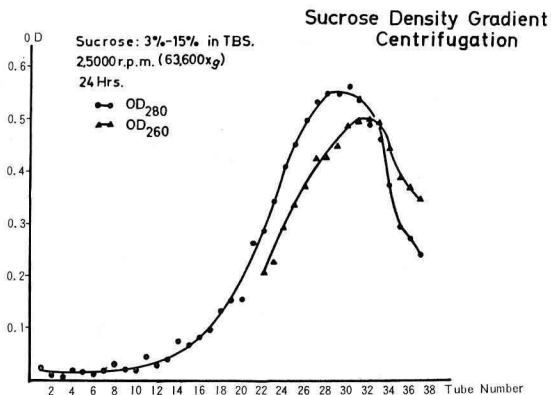

Fig. 7. Optical density curve of T–SFII after sucrose density gradient ultracentrifugation; I.

in which linear gradient of sucrose from 3% to 15% (W/V) in 1/20M-tris-buffered saline was used and spun at 25,000 r. p. m. (63,600 x g) for 24 hours. Figure 8 is another case in which the sucrose gradient was from 3% to 13% and
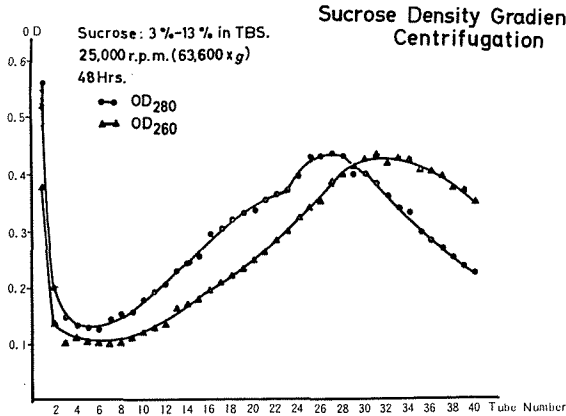

Fig. 8. Optical density curve of T-SFII after sucrose density gradient ultracentrifugation; II.

was spun at the same speed for 48 hours. From these data, protein and ribonucleic acid amounts were estimated (Fig. 9). Each fraction was collected into two groups; one, fraction I, consisting of tube numbers 1 to 10 and the other, the fraction 11, 13 to 31 (Fig. 9). After being concentrated to the equal protein
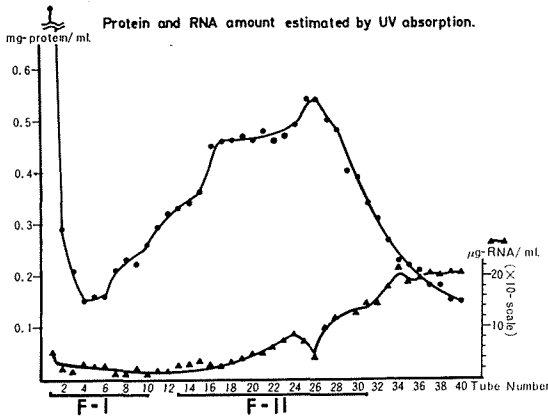
Fig. 9. Protein and RNA amount estimated by optical density curve of Figure 8.

F-I: fraction I in the text.
F-II: fraction II in the text.

amount to each other, each fraction was tested against anti-K45 in agar gel. Fraction I revealed all "kidney-specific" and non-specific precipitin lines, whereas no antigen was detectable in fraction II, which involves the major peak of protein.

Figure 10 shows the result when absorbed anti-K45 was used, and revealed a presence of "kidney-specific" antigens in fraction I. In order to confirm the
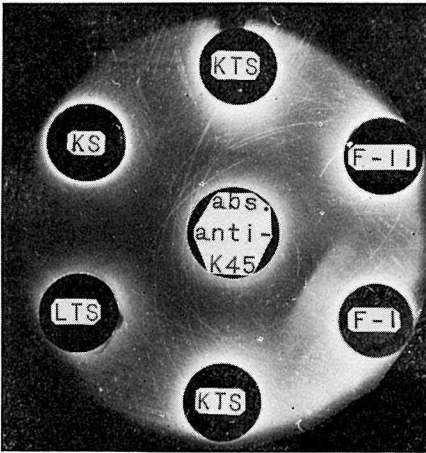
Fig. 10. Serological reactivity of F-I and
F-II.

abs. anti-K45: anti-K45 serum absorbed
with KS, LTS and chick-
en serum.

KS: postmicrosomal supernatant from
chicken kidney.

LTS: TS-fraction from chicken liver.

F-I: fraction I (tube numbers 1-10) after
density gradient ultracentrifuga-
tion.

F-II: fraction II (tube numbers 13-31).

Table 3.   Serological reactivity of the test antigen samples against "kidney-
specific" antiserum (absorbed anti-K45) by ring precipitin test.

Series of dilution* of test antigens.

	x 1**	x 3	x 9	x 27	x 81	x 243
Chicken						
Kidney TS	+	+	+	+	−	−
S	−	−	−	−	−	−
F-I	+	+	+	+	±	−
F-II	±	−	−	−	−	−
Serum	−	−	−	−	−	−
Liver TS	−	−	−	−	−	−
S	−	−	−	−	−	−
Quail						
Kidney TS	−	−	−	−	−	−
S	−	−	−	−	−	−
Mouse						
Kidney TS	−	−	−	−	−	−
S	−	−	−	−	−	−
Control ***	−	−	−	−	−	−

* Each test antigen sample was diluted with tris-buffered saline.

** Each antigen sample in this column contained 200 µg-protein per milliliter.

*** Tris-buffered saline only.

TS: TS-fraction of microsomes. S: postmicrosomal supernatant dialysed against
tris-buffered saline. F-I: fraction I (tube numbers 1 to 10) of the T-SFII of the
chicken kidney microsomes after sucrose density gradient ultracentrifugation.
F-II: fraction II (tube numbers 13 to 31) of the same original sample mentioned
above.

See the text for further details.

"kidney-specific" reactivity of the centrifugally separated fraction, ring precipitin test was done against absorbed anti-K45 antiserum. The latter was prepared by adding D- or TS-fraction from chicken livers. Protein concentration of each antigen solutions was adjusted equally to each other. The results are shown in Table 3. Absorbed anti-K45 antiserum which is highly "kidney-specific" reacts against fraction I (F-I in Table 3) with higher titer than against TS-fraction from kidney microsomes.

Ultracentrifugal Analysis

Subfraction T-SFII from kidney microsomes was analysed by analytical ultracentrifuge, Hitachi UCA-1 (Hitachi Ltd. Tokyo, Japan). The experimental conditions are in the legend to Fig. 11. Six minor peaks were followed with one major peak (M). The sedimentation coefficients of the minor peaks are calculated to be 6.0 S, 6.9 S, 7.7 S, 9.9 S, 12 S and 15 S respectively. The sedimentation coefficient of the major peak is approximately 2.1 S. Partial separation of the
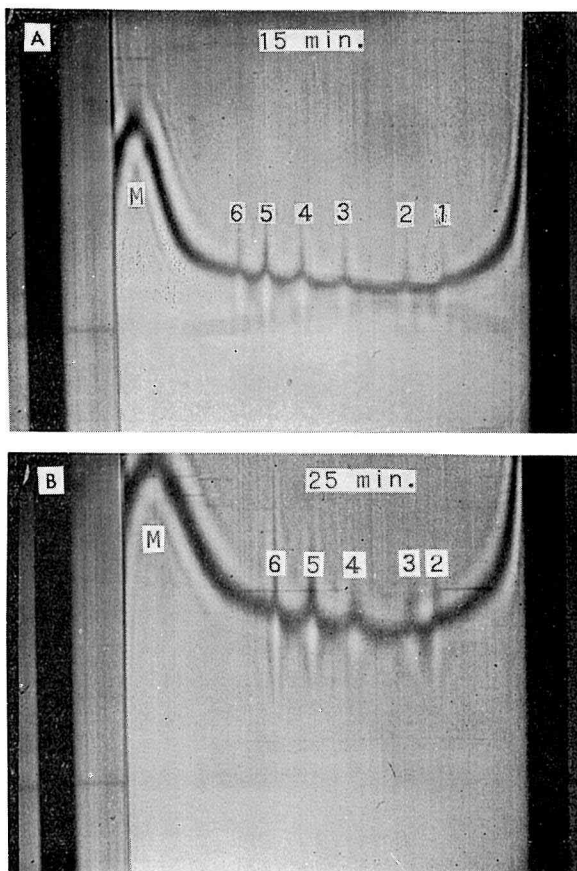

Fig. 11.   Sedimentation pattern of T-SFII.

The sample was spun at 60,000 r. p. m. at 20°C.
A. The photograph was taken at the 15th minute with the slit angle of 58 degrees.
B. The photograph was taken at the 25th minute with the slit angle of 60 degrees.

1-6: minor components in the text.
M: major component in the text.

minor components from the major one was attempted using a fixed septum–
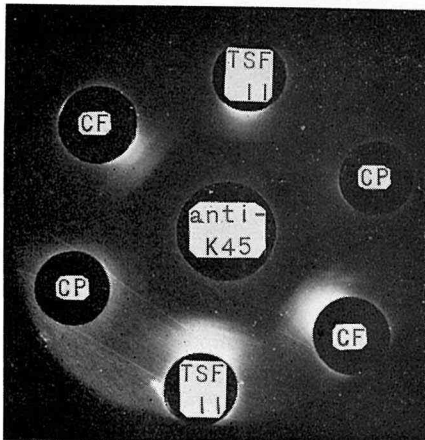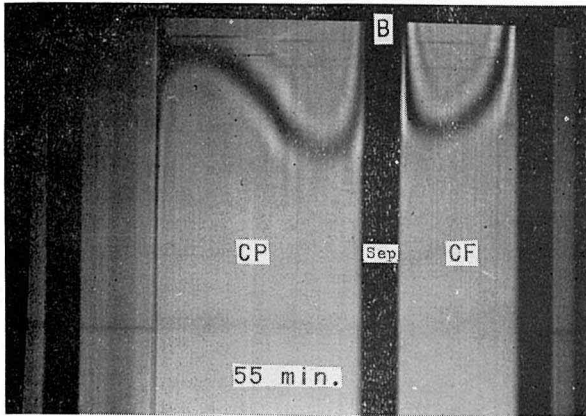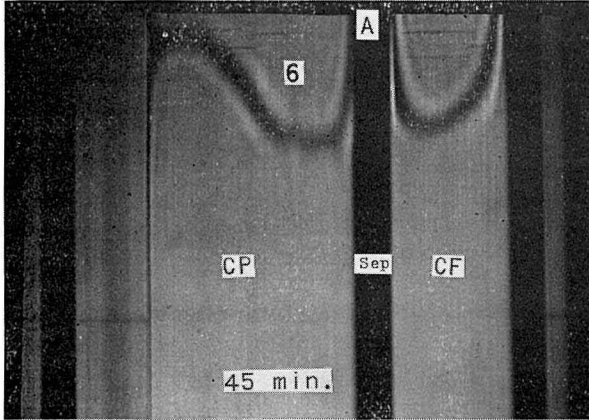

Fig. 12. Partial separation of minor components from major one by analytical ultracentrifuge.

The sample was spun at 60,000 r. p. m. at 20°C.

6: the 6th minor component seen in Figure 11.

Sep: small pitted septum.

CF: centrifugal half.

CP: centripetal half.

A. The photograph was taken at the 45th minute with slit angle of 60 degrees. The 6th minor component is still in the centripetal half.

B. The photograph was taken at the 55th minute with the slit angle of 60 degrees. The 6th minor component has already run away from the centripetal half.

Fig. 13. Serological reactivity of the fractions partially separated by analytical ultracentrifuge.

CF: centrifugal half.

CP: centripetal half.

Each antigen solution contains 2 milligrams of protein per milliliter.

See Figure 12 also.

separation cell (Hitachi Ltd. Tokyo, Japan). In the middle course of centrifugation when the minor components just passed through the small pitted septum in the cell (Fig. 12), the sample was took out into two fractions. The centripetal half (CP) contains the major component only, whereas the centrifugal half (CF) contains both the minor and major peaks. When these two with equal protein concentration were tested against anti–K45 in agar gel (Fig. 13), all the antigens were detected in CF but none in CP.

Interspecific Distribution of Kidney Microsomal Antigens

In order to survey the interspecific distribution of the antigens, kidneys from mouse and from Japanese quail, *Coturnix coturnix japonica*, were tested for immunological tests *in vitro*. Postmicrosomal supernatant and TS–fraction from mouse and from quail kidneys were prepared in the similar manner to the chicken materials. In the reaction against anti–K45 serum, postmicrosomal supernatant (S) of quail kidneys revealed as many and clear precipitin lines as that seen in chicken kidneys (Fig. 14). TS–fraction from quail kidneys produced some of the lines commonly seen in S and these were considered to be neither "microsome–
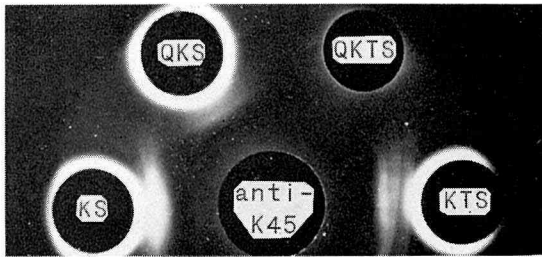

Fig. 14. Interspecific distribution of kidney microsomal antigens; I.
QKTS: TS-fraction from Japanese quail kidney.
QKS: postmicrosomal supernatant from Japanese quail kidney.
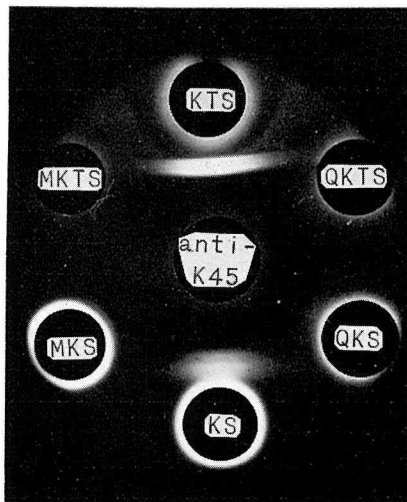Each antigen solution contains 2 milligrams of protein per milliliter.

Fig. 15. Interspecific distribution of kidney microsomal antigens; II.
MKTS: TS-fraction from mouse kidney.
MKS: postmicrosomal supernatant from mouse kidney.
Each antigen solution contains 2 milligrams of protein per milliliter.

specific" nor "kidney-specific" in respect with the chicken materials (18, 21) (Fig. 14). The line common to "kidney-specific" antigens of chicken was not detectable in quail kidneys.   The result of ring precipitin test against absorbed anti-K45 was also negative (Tab. 3).   The postmicrosomal supernatant and TS-fraction from mouse kidney did not react against anti-K45 both in Ouchterlony test (Fig. 15) and in ring precipitin test (Tab. 3).

Discussion

Judging from the procedures for fractionation, our D-fraction which is a DOC-soluble part of the microsomes, must contain lipoproteins constituting endo-plasmic reticulum as main components (21, 27).   All the antigens detected in the original D-fraction can be recovered in three different subfractions, SF15/25, SF25/45 and SF60/80, which are salted out by the different concentrations of ammonium sulfate.   Of these, only SF25/45 contains "kidney-specific" antigens, and this is characterized by high phospholipid-phosphorus content (20). The data given in Table 1, in which the salting out was done with somewhat different concentrations from those given above, may suggest that the corresponding subfraction from liver or lung microsomes (SF15/50) is also rich in phospholipid-phosphorus.   A distribution of "liver-specific" antigens in D-fraction has been shown in the cases of chicken and rat liver (24, 28, 29).   These findings suggest that "organ-specific" antigens in microsomes may be chemically very similar molecules among different organs of various higher vertebrates.

The "organ-specific" antigens in D-fraction from chicken kidney microsomes have been proved to be a good marker for tracing molecular changes in tissue differentiation at subcellular level.   The studies along this line has been per-formed in the course of embryonic development (22) and in the so-called "dedif-ferentiating" process in the monolayerly cultured cells (23).   A decrease of "liver-specific" antigens in D-fraction was also reported in the regenerating por-tion of partially hepatectomized rat liver (29, 31, 32), or hepatoma of rat induced with azodye (30, 31, 32).   Under these situations further characterization of the antigenic molecules with such specificity seemed to be necessary.   The molecules in discussion, according to our procedures previously established (20), were solu-bilized only after treatment with detergent, which will perhaps combine with the molecules contained in microsomes. Such situations render further purification and characterization of the "specific" antigens difficult.   Therefore, an effort to solubilize these antigens without any use of detergent was needed.

For this purpose removal of lipids from the washed microsomes was tried with organic solvents under proper conditions.   The results showed that after such treatment "kidney-specific" antigens can be made soluble in tris-buffered saline, preserving the original serological specificities.

Characterization of tris-buffered saline-soluble antigens (TS-fraction) was done by several techniques.   Results of Sephadex gel filtration show that one

peak (fraction A) contained all the molecules to be detected by Ouchterlony method.   Results of density gradient ultracentrifugation and ultracentrifugal analysis indicate that the major portion of TS-fraction from kidney microsomes does not contain the molecules to react with this antiserum (anti-K45) prepared against SF25/45 of kidney microsomes.   All the antigens mentioned in this paper are distributed in the minor components which sediment faster.   Why has the slowly sedimentable major component no serological reactivity as test antigens ? One possibility is that the rabbits did not recognize these molecules as antigens to elicit antibodies. The second is that, in the course of lipid extraction, a certain amount of the antigens are degraded into immunologically different proteins which are now recognized as the major component after density gradient ultracentrifugation.   Several peaks observed after ultracentrifugal analysis may present each serologically different antigens, but under the present experimental conditions it is difficult to collect the separated sample from each peak up to enough amount for immunological test *in vitro*.

The next Histocompatibility antigens which are considered to be membraneous lipoproteins (9) have recently been made soluble by use of proteolytic enzyme (6, 17), or by autolysis (17).   There is a suggestion that organ-specific antigens of microsomes are associated with the antigenic specificity for histocompatibility (25).

It has been reported that, in experimental allergy, phospholipid is responsible as a hapten for immunological organ specificity (11, 12, 13). But in the case of "kidney-specific" antigens reported in this paper, it can be postulated that the determinant group of immunological specificity may not reside in the phospholipid portion of the lipoproteins of the kidney microsomes, because in spite of the removal of more than 95% of phospholipid-phosphorus from the microsomes, "kidney-specific" antigens (together with non-specific ones) were detected in TS-fraction without any loss of immunological specificities.   Probably the antigens responsible for renal allergy reported by the previous authors may be different molecules from our present "kidney-specific" antigens.   This can be surmised from the informations of the histological distributions as well.   Our antigens were proved to localize in the epithelial cells of the proximal convoluting part of renal tubules (22), whereas experimental renal allergy was observed in glomerular tissue.

The next question to arise is that which of protein or polysaccharide will carry the determinant groups of immunological specificity.   The present result indicates that polysaccharides from kidney microsomes does not inhibit the reactivity of the antiserum.   From all these data, it may be speculated that the determinant groups of immunological "kidney-specificity" of our microsomal antigens may reside in the protein.

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