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Characterization of the Microsomal Antigen Related to a Subclass of Active Chronic Hepatitis

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Summary. The liver–kidney microsomal (LKM) antigen related to a subgroup of patients with active chronic hepatitis was studied biochemically and by immunoelectronmicroscopy, using peroxidase-conjugated antibodies. The antigenic determinant appears to be on a lipoprotein membrane since it required both phospholipid and protein for antigenic integrity and could not be solubilized.

Complement fixation and absorption of immunofluorescence with microsomal subfractions pointed to localization in both rough and smooth endoplasmic reticulum (ER). Immunoelectronmicroscopy on liver and kidney sections showed an association of the antigen with ribosomes and membranes in rough ER; granular staining was also seen between cisternae. Smooth membranes could not be preserved sufficiently to be recognized. The antigen is independent of ribosomal RNA.

INTRODUCTION

Microsomal autoantibodies reacting by immunofluorescence mainly with proximal renal tubules and hepatocytes, have been described in a clinically distinctive subgroup of patients having a chronic aggressive hepatitis, and in other disorders (Rizzetto, Swana and Doniach, 1973). These antibodies are quite difficult to distinguish from the mitochondrial antibodies associated with primary biliary cirrhosis (Walker, Doniach, Roitt and Sherlock, 1965; Doniach, 1972), since both stain kidney and liver. To separate the two immunofluorescence patterns it was necessary to use rat kidney sections which included cortex and medulla: both antibodies reacted variably with proximal tubules, but only mitochondrial antibodies stained ascending loops of Henle and distal tubules in the medulla. The two antigens were separated on the basis of quantitative complement fixation (CFT) and absorption studies with appropriate subcellular fractions (Rizzetto *et al.*, 1973).

In the present study the antigen was localized within subfractions of rat liver microsomes and its biochemical properties were investigated. Its distribution within the endoplasmic reticulum (ER) was visualized by immunoelectronmicroscopy with peroxidase-conjugated antibodies and the ultrastructural appearance of the antigen–antibody interaction was clearly distinguished from that of the mitochondrial primary biliary cirrhosis system (Bianchi, Penfold and Roitt, 1973).

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MATERIALS AND METHODS

Patients' sera

A standard serum from a 25-year-old female patient (M.B.) with active chronic hepatitis was used for all quantitative CFT studies. Her serum gave the microsomal fluorescence pattern on rat kidney and liver to a titre of 640; it also reacted with her own liver biopsy and fixed complement to 256 with rat liver microsomes. For the antigen studies, a constant serum dilution of 1:50 was selected. To ensure that the CF specificity was not confined to this particular patient, another serum from an ACH patient (MdB, kindly given by Dr J.-C. Homberg, Paris) with a similar fluorescence and CFT titre of 512 was used for studies on microsomal subfractions at a dilution of 1:50.

For fluorescence absorption experiments, sera from five patients with chronic hepatitis were used at dilutions between 20 and 50. A direct conjugate of the IgG from one of these patients was also employed at a 1:3 dilution. Sera from primary biliary cirrhosis patients containing high titre mitochondrial antibodies were tested similarly for comparison.

Tissue fractionation

Mitochondria and microsomes were prepared from rat liver and checked for degree of contamination by their marker enzymes, succinic dehydrogenase and glucose 6-phosphatase respectively, as described by Berg, Doniach and Roitt (1967). Thyrotoxic thyroid microsomes were prepared according to Roitt, Ling, Doniach and Couchman (1964). Subfractionation of liver microsomes into rough and smooth membranes was carried out by the method of Rothschild (1963). The 105,000 ***g*** microsomal pellet was resuspended in 2·0 M sucrose and covered with 15 ml aliquots of 1·5 M, 1·32 M and 0·25 M sucrose to form a discontinuous gradient. The tubes were spun in a SW 25-2 rotor for 15 hours at 25,000 rpm. Three isopycnic membrane layers and a pellet were obtained, each of which was checked for purity by electron microscopy (EM). Smooth membranes concentrated at the 0·25–1·32 M interface, rough membranes between the 1·5–2·0 M layers, and the pellet consisted of polyribosomes, variously contaminated with membranes. These three subfractions were used for CFT and absorption of immunofluorescence. The 1·32–1·5 M interface contained a mixture of smooth and rough membranes and was discarded.

Isolated ribosomes were prepared according to Tashiro & Siekevitz (1965). Microsomal suspensions in Tris Buffer, pH 7·6 with 0·001 M $MgCl_2$ were treated with sodium deoxycholate at a final concentration of 0·5 per cent. The suspension was shaken gently until cleared, spun at 105,000 ***g*** for 90 minutes and washed. Lastly 1·0 M $MgCl_2$ was added to a final concentration of 0·05 M to aggregate the ribosomes.

Immunofluorescence (IFL)

Standard tests were made on unfixed 5 μm cryostat sections of human thyrotoxic thyroid and stomach, and rat liver and kidney, while special experiments included pancreas, brain and other organs. FITC-conjugated anti-human IgG was mostly employed for the sandwich test. The following fixation methods were tried on rat kidney and liver sections: cold ether, ethanol and acetone for 10 and 30 minutes; 1 per cent paraformaldehyde; 1 and 2·5 per cent glutaraldehyde in 0·1 M phosphate buffer. These were also tried on 2 mm

blocks for use with EM. Unfixed and paraformaldehyde-fixed liver and pancreas sections were digested with Ribonuclease A (Sigma, type 1) dissolved in phosphate-buffered saline, pH 7, or normal saline and distilled water pH 6·5 at a concentration of 0·5 or 1·0 mg/ml for 1–2 hours at room temperature.

Complement fixation (CFT)

The liver microsomal subfractions were tested qualitatively by microtitre and active fractions assessed by quantitative CFT as described in Roitt *et al.* (1964), using the standard MB serum at 1:50. Thyroid microsomes and rat liver mitochondria were tested in parallel for comparison. Liver microsomes treated with chemicals and enzymes were similarly tested and compared with the untreated subfraction in each instance.

Absorption experiments

Fluorescence absorption studies were carried out as previously described (Rizzetto *et al.*, 1973). 0·2 aliquots of positive sera diluted twenty to fifty times were incubated overnight and stirred at 4° with an equal volume of whole microsomes, rough and smooth membranes, RNase-treated microsomes, isolated ribosomes, human thyroid microsomes and rat liver mitochondria respectively, with antigen concentrations ranging from 10 mg protein/ml in two-fold dilutions down to 0·03 mg protein/ml. Absorption of fluorescence was also attempted with detergent-solubilized microsomes but despite repeated dialysis residual detergent destroyed the sections.

Precipitin tests

Ouchterlony plates were made with agar or agarose (1–2 per cent in saline), PBS or veronal buffer. Plates containing the detergent used to solubilize the microsomal pellets were also set up.

Biochemical reagents and protein assays

Phospholipase A (*Naja naja* venom), phospholipase C (*Clostridium perfrigens*), wheat-germ lipase (all from Koch Light Company), four-times crystallized chymotrypsin (BDH), trypsin (Boehringer), taka-diastase extracted from *Aspergillus oryzae* (Parke-Davies), ribonuclease A, Type I and B, Type III (Sigma), Triton X-100 (Lennig Chem), nonidet P40 (BDH), and sodium deoxycholate (Merck).

Protein estimations were carried out following the method of Lowry, Rosebrough, Farr and Randall (1951).

Physical, chemical and enzyme treatments were carried out as shown in Tables 1 and 2.

Immunoelectronmicroscopy

IgG isolated on DEAE columns from two patients with high titre microsomal antibodies (MB and TS) were conjugated with peroxidase (Px) as described by Petts and Roitt (1971) with a ratio of 5 mg protein to 15 mg peroxidase (Avrameas & Terninck, 1971). Normal IgG similarly conjugated served as a control. Rat liver and kidney fixed in 1 per cent formaldehyde freshly prepared from paraformaldehyde, were used as substrates. Kidney blocks were fixed by dipping for 40 minutes, and liver by perfusion. Twenty-micron cryostat sections were incubated in the Px conjugate and prepared for EM as for the mitochondrial system (Bianchi *et al.*, 1973), since both antigens were found

TABLE 1

EFFECT OF PHYSICAL AND CHEMICAL TREATMENT ON MICROSOMAL ANTIGEN

Treatment	Volume (ml) microsomal suspension 25 mg protein/ml	Experimental conditions	Percentage loss of antigen	Percentage recovery of antigen	
				Pellet	Supernatant
Ultrasonication	1	2 minutes 20 Kcs	0	100	0
Heating 100°	1	2 minutes	100	0	0
56°	1	15 minutes	86	14	0
		30 minutes	90	10	0
37°		2 hours	25	75	0
Acid	2	2 ml 0·01 N HCl pH 3·2 then 6·2	48	52	0
Acid glycine buffer	2	0·1 M glycine/HCl, pH 2·7 neutralized with 0·2 N NaOH after 5 minutes	87	13	0
Alkali	2	2 ml 0·01 N NaOH pH 10. neutralized with HCl after 5 minutes	59	41	0
EDTA	2	9 ml 0·66 M, pH 7·6, 1 hour, 37°, dialysed overnight	1	99	0
Urea 6 M	1	3 ml 8 M urea-dialysed	100	0	0
Urea 8 M	1	against 8 M urea	100	0	0
Hypotonic shock	1	9 ml 0·001 M phosphate. buffer, pH 7·6. NaCl added after 30 minutes at 4°	1	99	0

TABLE 2

EFFECT OF ENZYMES ON MICROSOMAL ANTIGEN

Enzyme	Enzyme concentration mg/ml*	Percentage loss of antigen	Percentage recovery of antigen (CFT)	
			Pellet	Supernatant
Trypsin †	0·2	50	50	0
α-chymotrypsin	0·3	31	69	0
Lipase	7	0	100	0
Phospholipase A	2	99	0	0
Phospholipase C	2	48	52	0
Taka-diastase	3	23	77	0
Ribonuclease A	1	32	68	0
Ribonuclease B	1	18	82	0

* 1 ml of enzyme dissolved in CFT buffer plus 1 ml microsomal suspension, 50 mg protein/ml, incubated at 37° for 2 hours in CFT buffer.

† 0·2 mg soya bean trypsin-inhibitor added after incubation.

to require the same conditions. These sections were examined without further staining on unsupported grids in an AEI EM 6B microscope at 50 KV.

RESULTS

CFT WITH SUBMICROSOMAL FRACTIONS

The two strongly positive sera MB and MdB both reacted with rough and smooth membranes and Table 3 shows the microtitre CFT comparing microsomal subfractions. The experiments were carried out twice with separate sets of subfractions. The highest

antigen concentration was in the rough ER on both occasions but smooth membranes were almost as active and this could not be attributed to contamination with rough membranes as judged by the EM appearance of the preparation. The untreated polysomal pellet was substantially less active than either smooth or rough membranes and there was a significant contamination with rough ER as seen by electron microscopy. Isolated ribosomes prepared from whole microsomal fraction by treatment with 0·5 per cent deoxycholate (Tashiro and Siekevitz, 1965) were practically inactive and this was not unexpected since this detergent destroyed the antigen. Commercial rat liver microsomal RNA, thyroid microsomes and rat liver mitochondria gave no fixation with the active chronic hepatitis sera. Results of quantitative CFT were in agreement since 1 CH_{50} unit was fixed by 65 μg protein/ml of rough membranes and 120 μg of smooth membranes. The polysomal pellet of the same gradient was tested at concentrations up to 500 μg protein/ml but no appreciable complement fixation occurred.

ABSORPTION OF IMMUNOFLUORESCENCE

With serum MB diluted 1:50, kidney and liver IFL was abolished after absorption with microsomal rough membranes at a concentration of 2 mg protein/ml while 3·4 mg were needed when absorbing with smooth membranes. RNase-digested microsomes were as active as the untreated fraction and absorbed the fluorescence to the same degree. Human thyroid microsomes left the liver/kidney fluorescence unchanged.

Purified ribosomes (5 mg protein/ml), commercial RNA from rat liver microsomes (25 mg/ml) and mitochondria (10 mg/ml) caused no decrease of positive staining.

Comparable results were obtained with the other four positive sera examined. A primary biliary cirrhosis serum containing mitochondrial antibodies was not affected after absorption with the same concentrations of microsomal subfractions, but became negative after incubation with 2·5 mg of purified mitochondria/ml.

DETERGENT AND SOLVENT TREATMENT

After treatment of the microsomal pellet with surface active agents the following fractions were examined: (1) pellet, dialysed in 0·5 M saline and tested by CFT; (2) supernatant in detergent, assessed for antigenicity by precipitin test with and without detergent; (3) precipitate after dialysis of supernatant in 0·5 M saline and centrifugation at 105,000 ***g*** for 1 hour; (4) final supernatant in aqueous solution, the latter two fractions assayed by CFT.

The percentage protein recovered in each fraction and the loss of antigen after detergent and solvent treatment are shown in Table 4. All detergents caused complete loss of antigenicity except for Triton X-100 which left 15 per cent of CF activity, recovered in the pellet. Pseudo-precipitin lines were obtained between one positive serum (MB) and Triton supernatant, but this could not be repeated with the IgG fraction from the same serum. All other patients' sera were negative. Of the solvents, all destroyed the antigen except acetone, after which 7 per cent of antigen activity was recovered in the pellet (Table 5).

CHEMICAL AND PHYSICAL TREATMENTS

Results and experimental conditions are shown in Table 1. The antigen was still active

TABLE 3

MICROTITRE CFT RESULTS WITH SUBFRACTIONS OF RAT LIVER MICROSOMES

Antigen concentration mg protein/ml	Antigen subfractions											
	Smooth ER		Rough ER		Polysomes		Ribosomes (desoxycholate-treated)		Ribosomal RNA		Whole microsomes	
2·50	4	4	4	4	4	4	3	3	0	0	4	4
1·25	4	4	4	4	4	4	0	3	0	0	4	4
0·60	4	4	4	4	4	4	0	0	0	0	4	4
0·30	4	4	4	4	3	4	0	0	0	0	4	4
0·15	4	4	4	4	0	0	0	0	0	0	4	4
0·07	0	2	4	4	0	0	0	0	0	0	4	2
0·03	0	0	0	2	0	0	0	0	0	0	0	0
0·01	0	0	0	0	0	0	0	0	0	0	0	0
	MB *	MdB *	MB	MdB	MB	MdB	MB	MdB	MB	MdB	MB	MdB

* Sera MB and MdB both used at 1:50 dilutions with all fractions.
CFT grading: 4 = 0 per cent haemolysis; 3 = 25 per cent haemolysis; 2 = 50 per cent haemolysis; 0 = 100 per cent haemolysis.
ER = endoplasmic reticuuim.

TABLE 4

EFFECT OF DETERGENTS ON LIVER/KIDNEY MICROSOMAL ANTIGEN

Detergent initial concentration percentage	Experimental conditions	Final concentration of detergent (per cent)	Percentage of protein recovered		Percentage of protein recovered after removal of detergent by dialysis	Percentage of CF activity	
			Pellet	Supernatant		Pellet	Dialysed
Deoxycholate (DOC) 1 dissolved in minimal amount of 0·1 N NaOH, to pH 7·6 with 0·1 N HCl, diluted in 0·1 M Tris HCl, pH 7·6	5ml + 5 ml microsomes (10 mg protein/ml) Shaken at 4° until clear, and immediately spun at 105,000 *g* for 90 minutes	0·5	13	87	6	0	0
DOC 0·5 (as above)		0·25	22	78	7	0	0
DOC 0·25 (as above)		0·125	28	72	9	0	0
Triton 0·5 dissolved in Tris HCl, 0·1 M, pH 7·6		0·25	20	80	14	Trace	0
Triton 0·2 dissolved in Tris HCl, 0·1 M, pH 7·6		0·1	34	76	10	15	0
Nonidet 2 dissolved in Tris HCl, 0·1 M, pH 7·6		1·0	26	74		0	0
Nonidet 1 dissolved in Tris HCl, 0·1 M, pH 7·6		0·5	42	58		0	0

TABLE 5

EFFECT OF ORGANIC SOLVENTS ON MICROSOMAL ANTIGEN

Solvent *	Treatment	Percentage loss of antigen
Ether, 1 ml	Shaken 1 minute RT in Whirlimixer	99
Butanol, 1 ml	Shaken 1 minute RT in Whirlimixer	99
n-Hexane, 1 ml	Shaken 1 minute RT in Whirlimixer	99
Chloroform, 2·66 ml + methanol, 1·4 ml	Shaken 30 seconds in Whirlimixer	99
Acetone, 2 ml	10 minutes at −5°	93
Ethanol, 7 ml	10 minutes	99

* 1 ml of microsomal suspension containing 50 mg protein used in all experiments. Samples spun at 105,000 *g* in the cold, supernatant dialysed against PBS, pellet and supernatant assayed by CFT.
RT = room temperature

after treatment with 0·01 N HCl. However, the pH of the suspension did not fall below 3·2 and returned to 6·2 at the end of the experiment, probably owing to the buffering capacity of the membranes. When an acid–glycine buffer was used and the pH maintained at 2·7, only 20 per cent of the antigen was recovered after 5 minutes. EDTA buffered to pH 6·5 did not affect the antigen and all the activity was recovered in the pellet. After alkali treatment 40 per cent was recovered but 6 M and 8 M urea caused complete inactivation. No loss occurred after hypotonic shock or ultrasonication. The antigen was heat-sensitive: it was destroyed by boiling, and almost inactivated at 56° for 30 minutes but 75 per cent of the activity was recovered in the pellet after heating at 37° for 2 hours.

EFFECT OF ENZYMES

Results are shown in Table 2. Phospholipase A destroyed the antigen completely, while phospholipase C caused a 50 per cent loss and lipase had no effect. Ribonucleases A and B had only slight effects and proteolytic enzymes reduced the activity by about half.

IMMUNOELECTRONMICROSCOPY

The peroxidase conjugate penetrated only the outer 3–4 μm of the cryostat sections. Under the experimental conditions used, nuclei, mitochondria and rough ER were fairly well preserved but it has not yet proved possible to visualize smooth ER. In liver sections the microsomal antibodies produced a dotted staining on the outer aspect of the rough ER, suggestive of ribosomal localization. With normal Px-conjugated IgG, the ribosomes were smaller and appeared as faint ghosts (Fig. 1). At the level of resolution achieved, it was not possible to say whether the antibodies also reacted with the ER membranes.

In the P_3 segment of renal proximal tubules the ER was more difficult to resolve, but again the staining produced by the antibodies had a dotted appearance. In some places short segments of rough ER could be distinguished and elsewhere clusters or isolated ribosomes appeared to take up the Px conjugate. With normal IgG the ER was very faint (Fig. 2). Nuclei, mitochondrial inner and outer membranes, plasma membranes and microvilli were unstained in both liver and kidney.

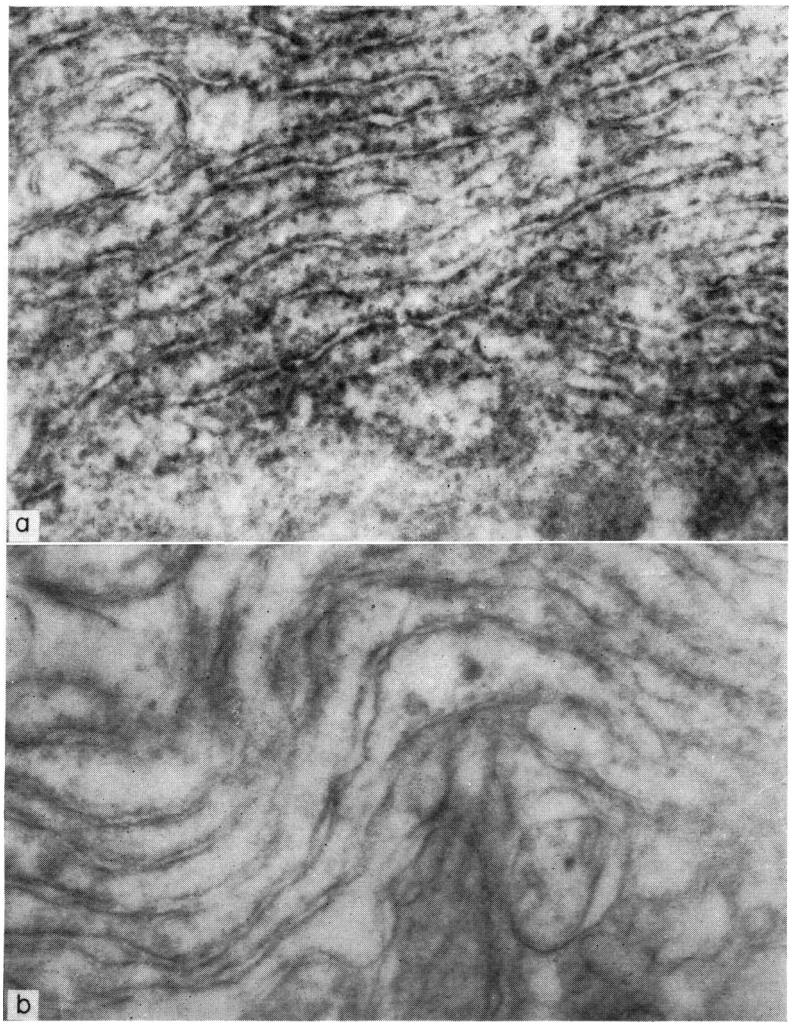

FIG. 1. (a) Rat liver cell treated with peroxidase-conjugated IgG from patient with active chronic hepatitis having LKM antibodies. ER show granular staining on outer aspect of membranes, suggestive of ribosomal localization. Mitochondrion at top left corner and portion of nucleus at lower edge are unstained. (Magnification × 24,000.) (b) Rat liver cell treated with peroxidase conjugate of normal IgG. Ribosomes are smaller and appear as faint ghosts. (Magnification × 24,000.)

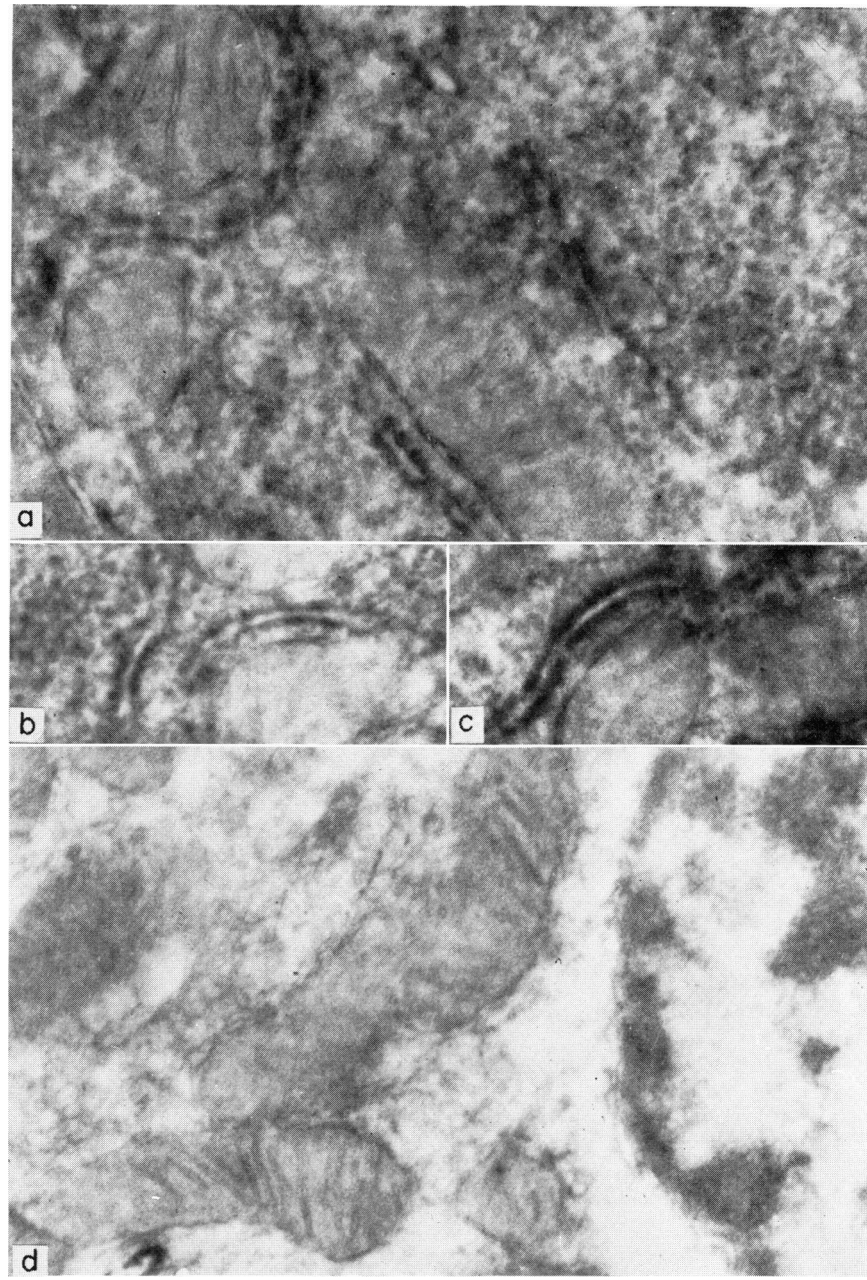

FIG. 2. (a) Rat kidney, lower proximal tubule (P_3) cell treated as in Fig. 1a. Microsomal LKM antibodies outline three rough ER cisternae close to mitochondria. Background granular staining suggestive of reaction with free ribosomes. (Magnification × 27,000.) (b) P_3 cell treated as in Fig 1a. Profiles of two ER cisternae stained with LKM antibodies. (Magnification × 27,000.) (c) P_3 cell showing heavy linear deposit of Px-conjugated antibody on ER cisterna, suggesting high concentration of LKM antigen in these cells (Magnification × 27,000.) (d) P_3 cell treated with Px conjugate of normal IgG. ER membranes near mitochondria and background show no staining. Portion of nucleus on right. (Magnification × 18,000.)

DISCUSSION

The microsomal antigen–antibody system discussed in this paper reacts in immunofluorescence mainly with liver and kidney, and although faint reactions were also obtained in some other organs, it was decided to call it 'liver–kidney microsomal' (LKM), to avoid confusion with the organ-specific antigens localized to closely related subcellular fractions. The chemical behaviour of LKM antigen shares certain features with the other tissue autoantigens analysed so far, i.e. thyroid (Roitt *et al.*, 1964), gastric (Baur, Roitt and Doniach, 1965), adrenal (Goudie, McDonald, Anderson and Gray, 1968), organ-specific microsomal, and the mitochondrial inner membrane antigen reactive in primary biliary cirrhosis (Berg, Roitt, Doniach and Horne, 1969a; Berg, Roitt, Doniach and Cooper, 1969b). All these antigens were inactivated by solvents and surface active agents and were sensitive to proteolytic enzymes. Thyroid and gastric parietal cell antigens are less sensitive to phospholipases than were the mitochondrial and LKM antigens. Phospholipase A inactivated these completely while phospholipase C caused a 50 per cent loss of LKM activity. Lipase, RNase and takadiastase on the other hand had no effect on any of the tissue antigens, except possibly adrenal. The antigens were more sensitive to acid than to alkali and were inactivated by heating. Osmotic shock, ultrasonication and EDTA had no substantial effect on any of the tissue antigens. Partial solubilization in 6–8 M urea proved possible in the case of the gastric antigen (Ward & Nairn, 1967) and with the mitochondrial antigen (Ben-Yoseph, Shapira and Doniach, 1974), but this treatment led to loss of LKM antigen and so far this has remained insoluble in its active form.

The question of the exact ultrastructural localization of the LKM antigen raises interesting problems. The beaded electron microscopic appearance with peroxidase-conjugated antibody on the rough ER of rat liver would suggest that the antigen is connected with ribosomes. The cytoplasmic areas where smooth ER is supposedly interspersed with free ribosomes also showed dotted staining and some of these dots were the size of free ribosomes. No clearly visible smooth ER was seen either in antibody-treated or control sections and it is probable that these delicate membranes are fragmented by the rough treatment they receive during cryostat sectioning, fixation and staining with Px IgG. When intracellular membranes are disrupted *in vitro* they always reaggregate in micellar vesicles which would be larger than free ribosomes but the fate of broken ER membranes in relatively intact cells is not known and no vesicles could be recognized in our preparations.

Complement fixation and fluorescence absorption results show that the antigen content of rough ER is only twice that of the smooth membrane subfraction while contamination did not exceed 10–20 per cent. Furthermore the sedimented polysomal subfraction was less active than the smooth ER. The fact that purified ribosomes were totally inactive could be due to the destructive action of deoxycholate used in their preparation.

In the kidney there is hardly any endoplasmic reticulum in the P_1 and P_2 portions of the proximal tubules (Beard & Novikoff, 1969), while in the P_3 portion it is more abundant and consists mainly of rough membranes (Maunsbach, 1966). The staining with Px-conjugated antibody was similarly negative in P_1 and P_2 and was dense in P_3, giving both a finely dotted background between mitochondria and a substantial thickening of the rough ER which was linear in places (Fig. 2b and c). Nevertheless, although the LKM antigen follows the distribution of rough ER in liver and kidney, other cells noted for their diffuse content of rough ER membranes such as thyroid, gastric chief cells,

exocrine pancreas, Betz cells in cerebral cortex, remained practically unstained by these microsomal antibodies in immunofluorescence. Therefore the antigen is not a general constituent of rough ER.

The antigen is certainly not connected with the RNA portion of the ribosomes since digestion with RNase did not diminish the antigenic potency of microsomes in CFT or in the ability of antigen to combine with antibody when the enzyme was applied directly to tissue sections. In addition EDTA and alkali treatment which are known to disrupt ribosomal RNA (Tashiro and Siekevitz, 1965) had no effect on antigenic activity.

It can be concluded that the LKM antigen requires both protein and phospholipids for antigenic integrity, as has been shown for other tissue antigens. It is probably localized in both rough and smooth ER. In the rough membranes, it seemed associated with ribosomes and membranes. Further experiments are required to find out the exact relationship to these structures. The diffuse fluorescence in liver cell cytoplasm favours both smooth and rough ER localization, especially in view of the existence of another antibody found mainly in patients with collagen disorders, which gives a perinuclear, coarsely granular fluorescence in hepatocytes (Doniach, Lindqvist and Berg, 1971) that can be absorbed out with purified ribosomes (Homberg, Rizzetto and Doniach, unpublished observations).

Knieser & Jenis (1972) described peroxidase immunoelectronmicroscopy with antibodies derived from patients with chronic liver disease, giving a staining pattern not unlike that produced by LKM antibodies. However, the organs shown in the illustrations of these authors are pancreas and gastric glands which react hardly at all with LKM sera. It is doubtful that these authors had classical primary biliary cirrhosis sera at their disposal since these have been shown conclusively to contain inner mitochondrial membrane antibodies by the same EM method (Bianchi *et al.*, 1973). Preliminary immunoelectronmicroscopy experiments with dispersed microsomal subfractions stained with Px-conjugated antibodies have so far proved unsuccessful in our hands for technical reasons and are being pursued.

From the clinical point of view it is of interest that two microsomal antibodies having a different organ distribution in immunofluorescence but both related in some way to proteins of the ER not involving RNA, should be polarized in patients with different autoimmune disorders. The LKM antibody appears to select out a subgroup of active chronic hepatitis patients which differ in their sex and age distribution and in somo serological features from other known varieties of 'autoimmune' liver disease (Rizzette *et al.*, 1973) while the ribosomal antibody has now been found in eight patients all suffering from systemic lupus erythematosus or closely allied collagen disorders (Homberg *et al.*, unpublished observations). The relation of these different tissue antibodies to the underlying cause of the respective clinical syndromes remains to be discovered.

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