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MICROSOMAL ANTIBODIES IN ACTIVE CHRONIC HEPATITIS AND OTHER DISORDERS

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SUMMARY

An autoantibody reacting with microsomal membranes has been characterized by a distinctive immunofluorescence pattern on proximal renal tubules and hepatocytes. The microsomal nature of the antigen was demonstrated by absorption and quantitative complement fixation studies. These results showed the antibodies to be quite distinct from the mitochondrial antibodies found in primary biliary cirrhosis.

Microsomal antibodies have so far been detected in sixteen cases, of whom twelve had liver disorders. These antibodies, although rare, may provide a serological marker for a small proportion of active chronic hepatitis cases differing in several respects from other recognized subgroups in this disease.

INTRODUCTION

Complement fixation studies with microsomal subcellular tissue fractions were reported by several authors following the work of Mackay & Gajdusek (1958), in systemic lupus erythematosus, chronic active hepatitis (ACH) associated with persistent autoimmunity (lupoid) and in primary biliary cirrhosis (PBC). At that time immunofluorescence (IFL) was rarely done and only three early reports mention liver cytoplasmic staining (Bumbalo, Bellanti & Terplan, 1962; Sturgill & Carpenter, 1965; Tsuji, Ogawa & Kosaka, 1965). In one of these studies the antibodies were thought to be ribosomal as the complement-fixing activity could be abolished by RNase. We have been studying an antibody which gives a diffuse, finely granular cytoplasmic IFL on hepatocytes with a more irregular fluorescence on other tissues (Doniach, Lindqvist & Berg, 1971). These antibodies have been found in occasional cases of ACH and in a small number of sera from patients with other autoimmune disorders. In the present study we report further characterization of these antibodies and present evidence that the antigen is in the microsomal membranes.

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MATERIALS AND METHODS

Patients' sera

The present study is based on the sera of sixteen patients collected over the past 5 years. Since becoming aware of these antibodies some ten further reactions were observed that could fit into the pattern but which were too weak to study in detail. Approximately 7000 sera were tested each year, of which up to 20% were from patients with liver disorders. Some of the patients with microsomal antibodies were tested repeatedly over a period of several years, while others could not be obtained again. Sera from patients with primary biliary cirrhosis containing mitochondrial antibodies were used for comparison of IFL patterns and complement-fixing activity. Specimens were stored at −20°C for up to 4 years.

Tissue fractionation procedure

The method of De Duve *et al.* (1955) was followed. Wistar rats weighing 100–150 g were starved overnight and killed by cervical dislocation. Batches of 40 g of liver were homogenized to 1 : 10 w/v in 0·25 M sucrose. From the cytoplasmic extract after removal of nuclei, four fractions were obtained by differential centrifugation in sequence: (1) Mitochondrial fraction, 7000 ***g*** for 10 min; fraction washed up to six times to reduce contamination with microsomes. (2) Intermediate fraction, 26,000 ***g*** for 30 min. This was found to be a mixture and was tested only once. (3) Microsomal fraction 105,000 ***g***, 90 min, washed twice with PBS. (4) Supernatant. Concentrated in hypertonic sucrose, dialysed 48 hr in PBS, used fresh or lyophilized.

In one experiment rats were pretreated with 200 mg of Triton WR 1339 (Rohm & Haas Co., Philadelphia, Pa) for the preparation of purified lysosomes (Wattiaux, Wibo & Baudhin, 1963).

Marker enzymes

Succinic dehydrogenase, the mitochondrial marker, was measured according to Der Vartainen & Veeger (1964); glucose-6-phosphatase was used as a check for microsomes and was estimated according to De Duve *et al.* (1955). In one experiment lysozyme was measured to identify lysosomes (Shugar, 1952).

Protein assays

Subcellular fractions were compared quantitatively on the basis of their protein content as measured by the method of Lowry *et al.* (1951).

Complement fixation tests (CFT)

For qualitative tests the microtitre method was used with 2 MHD of complement, chessboard tests being carried out for comparison of different sera and subcellular fractions. Quantitative CFT was done as described in Roitt *et al.* (1964) and Berg *et al.* (1967). To compare different antigenic fractions, the optimum dilutions of the two best complement fixing sera were chosen from CH_{50} isofixation curves. Quantitative CFT was studied only with mitochondrial and microsomal membrane fractions, since preliminary microtitre results showed the supernatant to be inactive. The intermediate (fluffy) layer was tested qualitatively in case the unknown antigen might be concentrated in it, but preliminary results showed it to be less active than the microsomes and it was discarded.

Immunofluorescence and immunoperoxidase staining

For standard testing, unfixed 5 μm cryostat sections of human thyrotoxic thyroid and stomach, and rat liver and kidney were employed. Various other human and animal tissues were also tested for comparison with the standard organs and with the mitochondrial antibodies seen in primary biliary cirrhosis. One block of rat kidney was cut transversely in serial sections, from the cortex to the medulla and each section numbered for study of the staining pattern at different levels. These were compared with corresponding cortical and medullary blocks from a fresh operative specimen of human kidney. Sera were tested at 1:10, and the fluorescence titre was established with increasing dilutions until an endpoint was reached. In the sandwich technique conjugates of anti-sera to whole human γ-globulin, IgG, IgA, IgM and β_1C were employed.

A direct fluorescein isothiocyanate (FITC) conjugate of the ACH microsomal antibody was applied in conjunction with a PBC globulin containing mitochondrial antibodies conjugated with tetramethyl-rhodamine in order to study the overlap of the two antigens in tubules at different levels of the renal cortex and medulla by double exposure photography. Two direct conjugates of microsomal and mitochondrial antibodies were also prepared by the peroxidase method as described by Petts & Roitt (1971). The Leitz Orthoplan microscope fitted with incident UV illumination was used in these studies. Photographs were taken with the automatic camera and high speed Ektachrome film.

Organ-specific and other autoantibodies

IFL on human thyroid and stomach detected gastric parietal (GPC) and thyroid microsomal reactions; CFT with thyroid microsomes was also done for titration of these antibodies and for control of organ-specificity. Anti-nuclear (ANA) and smooth muscle antibodies (SMA) were detected by IFL on rat liver and kidney. Tanned red cell (TRC) agglutination was employed for the detection of thyroglobulin antibodies (Roitt & Doniach, 1969).

Fluorescence absorption studies

The four most reactive sera giving microsomal fluorescence patterns were used in these experiments. Aliquots (0·2 ml) of seven serial dilutions of microsomal and mitochondrial antigen fractions containing from 20·0 down to 0·3 mg protein/ml were mixed with an equal volume of 1:20 or 1:40 serum dilutions and the tubes kept on a shaker overnight at 4°C. After centrifugation the supernatants were tested by immunofluorescence on rat kidney and liver sections. With two high titre sera (1:600) it was necessary to repeat the absorption a second time to abolish the fluorescence. As a control, parallel aliquots of each serum were diluted to the same final concentration with PBS and incubated with the tests. A PBC serum containing mitochondrial antibodies was absorbed in the same way with both subcellular tissue fractions for comparison.

RESULTS

Microsomal immunofluorescence patterns

One of the characteristic features of the microsomal antibody was the uniform finely granular bright IFL seen throughout the cytoplasm of hepatocytes (Fig. 1). The pattern was

similar in liver from human, adult and foetal rat, pig, sheep and beef. No staining was seen on Kupffer cells or other interstitial elements, portal spaces being unstained up to the limiting plate. All the sera gave positive IFL on rat and human kidney to the same titre as with liver. The distribution of staining varied between human and rat kidney and in the latter also to a certain extent between different positive sera. In both kidneys the proximal tubules were most conspicuous and there was a complete absence of staining in distal tubules and ascending loops of Henle seen in the medulla (Fig. 2a and b). In the human

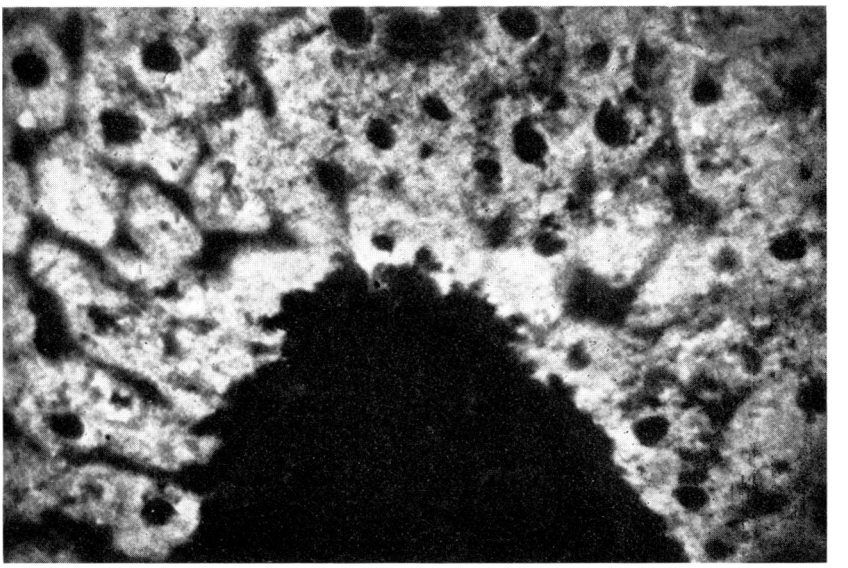

FIG. 1. Rat liver treated with 1:100 dilution of serum from a 13-year-old boy (JW) with active chronic hepatitis, containing microsomal antibodies, followed by FITC-conjugated rabbit anti-human γ-globulin. Hepatocytes show intense finely granular IFL. No staining of periportal connective tissue or Kupffer cells ($\times$ 280).

the entire cortex appeared stained whereas in the rat only the inner cortex showed fluorescence. In the outer cortex, wedges of stained tubules alternated with negative regions, the extent of these non-reactive areas varying between one serum and another irrespective of the antibody titre. With seven sera the reactive wedges reached the capsule while with nine others the outer cortex was unstained in the same kidney.

On human thyroid cytoplasm five sera showed strong IFL which was due to organ-specific thyroid microsomal antibodies since three of the patients also reacted by CFT with thyroid in titres of 16–64 and four had positive TRC indicating thyroglobulin antibodies in addition. Five of the sera showed a trace of staining and six were completely negative.

Human stomach gave even more variable results: one serum (CR) showed strong and equal fluorescence of parietal and chief cells: five sera produced a trace of GPC staining, eight were negative and two patients had strong organ-specific GPC patterns.

Two high titre sera were also applied to human brain, submaxillary gland, adrenal cortex and rat pancreas, heart and striated muscle. All these organs showed weak staining

with indefinite patterns with the anti-microsomal sera. These were quite different from the patterns obtained with PBC mitochondrial antibodies.

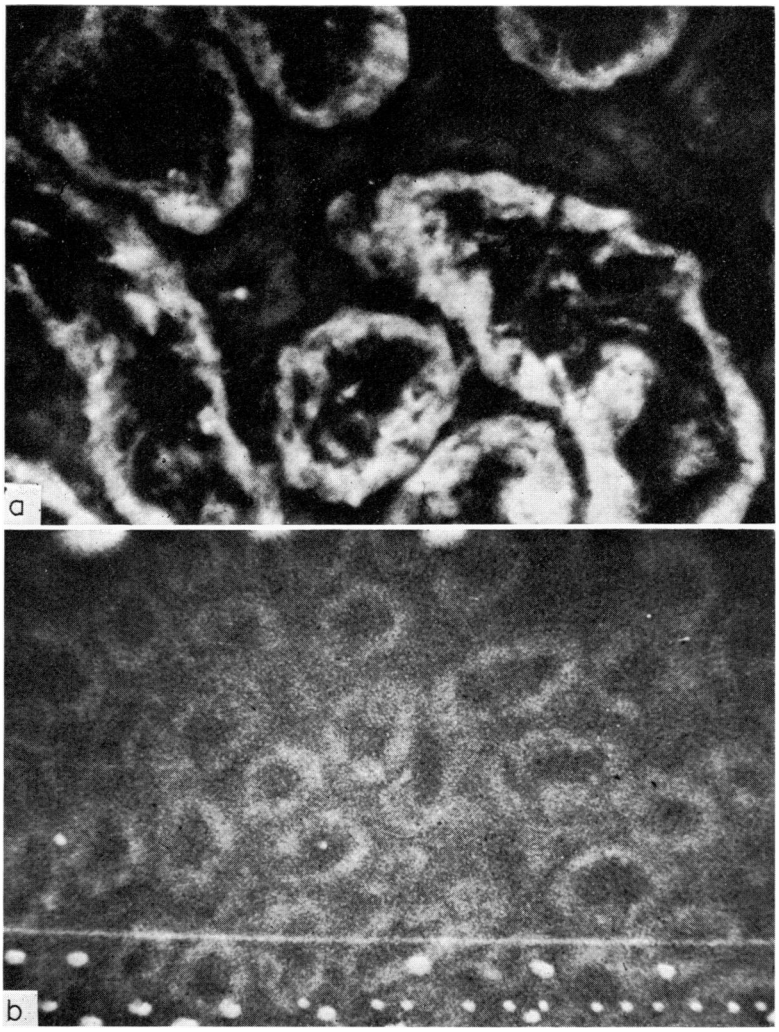

FIG. 2. (a) Rat kidney cortex treated with direct FITC conjugate of Ig from serum JW. The proximal tubule cytoplasm is uniformly stained. Distal tubules, ? loops of Henle, seen in centre, are unstained (× 280). (b) Rat kidney medulla treated as (a), showing absence of IFL in loops of Henle (× 175).

Diseases associated with microsomal antibodies

The clinical diagnoses are shown in Table 1. Of the sixteen sera studied in some detail, twelve were from patients with liver disorders: eight had an active form of chronic hepatitis (four males and four females). Two female patients were thought to have halothane jaundice, one man had proven liver cancer and one female patient had a multi-system autiommune

disorder which included chronic hepatitis with enlarged liver, raised alkaline phophatase and abnormal BSP retention, long-standing rheumatoid arthritis, a goitre with thyroid antibodies, and chronic false positive reactions for syphilis (BFP). One male patient presented an obscure neurological disorder with severe demyelination and subclinical liver disease with peri-portal lymphoid infiltrates. The remaining three cases were diagnosed as having Hashimoto goitre, vitiligo and symptomless chronic BFP reaction.

The eight cases of overt active chronic hepatitis could not easily be classified as 'autoimmune' in the usual sense, since all had negative ANA and no more than three showed a trace of SMA at 1:10 only. One man had Australia antigen in the serum on repeated testing for 3 years but all the other liver cases were negative for HB virus. Thyroid specific antibodies were present in five of the patients, four of whom had liver disease, and gastric parietal cell antbodies were detected in two cases. The incidence of organ-specific antibodies in this group of patients is thus higher than would be expected in unselected material.

TABLE 1. Clinical diagnosis in sixteen patients with microsomal antibodies in the serum

Diagnosis	Number of cases
Active chronic hepatitis	8
Halothane jaundice	2
Hepatoma	1
Subclinical hepatitis, RA and BFP	1
Chronic false positive Reaction for syphilis (BFP)	1
Unexplained neuropathy	1
Hypothyroidism and vitiligo	2
Total cases	16

Serological properties of positive sera

The titre of microsomal IFL varied from 1:40 to 1:800 in the sixteen sera described. As mentioned above, another ten sera gave weak reactions at 1:10 only and it is not certain whether these were truly microsomal in pattern. Eight of the sixteen patients were tested repeatedly over periods up to 5 years and none became negative. In some cases the titre remained unchanged. However, in one boy aged thirteen with active chronic hepatitis, the titres went down from over 600 to 40 over a follow-up period of 3 years during which there was also a gradual clinical remission. In another case of ACH a girl aged 17 (JA in Doniach *et al.*, 1971) the titre was 800 when first tested and gradually decreased to 200 while the clinical condition progressed to inactive cirrhosis over a period of 5 years.

The autoantibodies were predominantly IgG in fourteen sera and entirely IgM in the two patients who also showed chronic BFP reactivity. Fluorescence with anti-β_1C conjugate was positive in four of the seven sera with the highest titres. CFT in microtitre trays was positive with microsomal and mitochondrial fractions in the four sera fixing C on immunohistology. With mitochondria, CFT required far more antigen and results varied with the degree of microsomal contamination in different subcellular preparations. 'Supernatant'

soluble protein fractions gave negative results with all sera and 'fluffy layer' fixed less well than microsomes. For this reason all quantitative CF studies were pursued only with microsomal and mitochondrial fractions.

Enzyme markers

Mitochondrial and microsomal fractions were checked for contamination with each other by marker enzyme tests. On the basis of glucose 6-phosphatase estimations, mitochondrial fractions could be contaminated to the extent of 50% with microsomes. After six washes this was reduced to 10–30% in different experiments. Microsomal fractions were usually free of mitochondria as judged by succinic dehydrogenase estimations.

TABLE 2. Absorption of microsomal and mitochondrial immunofluorescence with rat liver subcellular fractions

Antigen used for absorption mg protein/ml	Immunofluorescence on rat kidney and liver: Serum JW 1:20 microsomal antibodies		Serum MS 1:20 mitochondrial antibodies	
	1st abs.	2nd abs.	1st abs.	2nd abs.
Microsomes				
20·0	−	−	+	+
10·0	−	−	+	+
5·0	±	−	+	+
2·5	+	−	+	+
1·25	+	+	+	+
0·6	+	+	+	+
0·3	+	+	+	+
Mitochondria				
20·0	+		−	
10·0	+		−	
5·0	+		−	
2·5	+		±	
1·25	+		+	
0·6	+		+	
0·3	+		+	

Lysozyme assay in one experiment gave positive results in all subcellular fractions with maximum reactivity in the mitochondrial pellet. In the special fractionation done with rats pre-treated with Triton to obtain better separation of lysosomes, the gradient band which contained the highest quantity of these particles, proved to be a poorer CFT antigen having less than 50% the activity of the corresponding microsomal membrane layer. Therefore lysosomes were not studied further.

Absorption of IFL by subcellular fractions

Absorption of four positive sera was carried out as described in the methods. The microsomal fraction absorbed the liver/kidney fluorescence in the four sera tested, while no effect

was obtained with highly purified mitochondria (six washes). A serum (MS) containing mitochondrial antibodies was absorbed in the same manner with the two fractions. In this case, fluorescence could be removed with mitochondria and not with microsomes. A representative experiment is shown in Table 2.

One microsomal serum was also absorbed with lyophilized supernatant fraction at the same protein concentrations and there was no diminution of IFL.

Quantitative CFT

CH_{50} isofixation curves could be established with only two anti-microsomal sera. The other available sera fixed insufficient C to be studied by this method. Serum MB (IFL 600) fixed C in serum dilutions between 1:8 and 1:128 with 20–60 μg protein/ml of microsomes while twenty times as much mitochondria were required to obtain a parallel curve (Fig. 3).

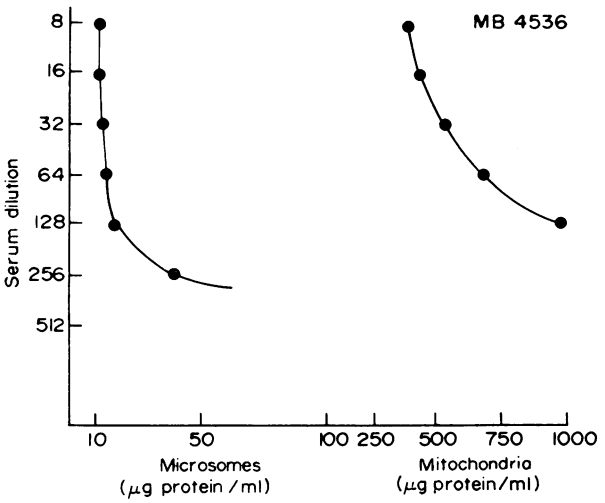

FIG. 3. CH_{50} isofixation curves of serum MB from 25-year-old female patients with active chronic hepatitis, containing microsomal antibodies. 20 μg protein/ml of microsomal antigen was required. The mitochondrial fraction was active at twenty times this concentration.

In this experiment the mitochondrial fraction was successfully purified and contained an amount of glucose 6-phosphatase indicating about 10% microsomes. Serum CR (IFL 200) was less sensitive to microsomal antigen, requiring more than 1000 μg protein/ml for isofixation in dilutions of 1:8–1:64. Three times more mitochondrial antigen was required. Glucose 6-phosphatase estimations showed a microsomal contamination of more than 30% in the mitochondria used on this occasion.

Comparison of microsomal and mitochondrial immunofluorescence

The two direct conjugates, anti-microsomal FITC and anti-mitochondrial-Rhodamine were applied together to serial sections of rat kidney to compare the topographical distribution of the two antigens. Double exposure photography showed that the ascending loops of Henle and distal tubules (LHD) contained only mitochondrial antigen (Fig. 4a) while

proximal tubules stained more strongly with microsomal antibody (Fig. 2a) and to a lesser extent also with anti-mitochondrial conjugate showing a mixture of red and green on coloured slides. Representative coronal sections were selected from 101 serials starting near the renal capsule and reaching down to the medulla. The number of tubules (LHD) strongly stained for

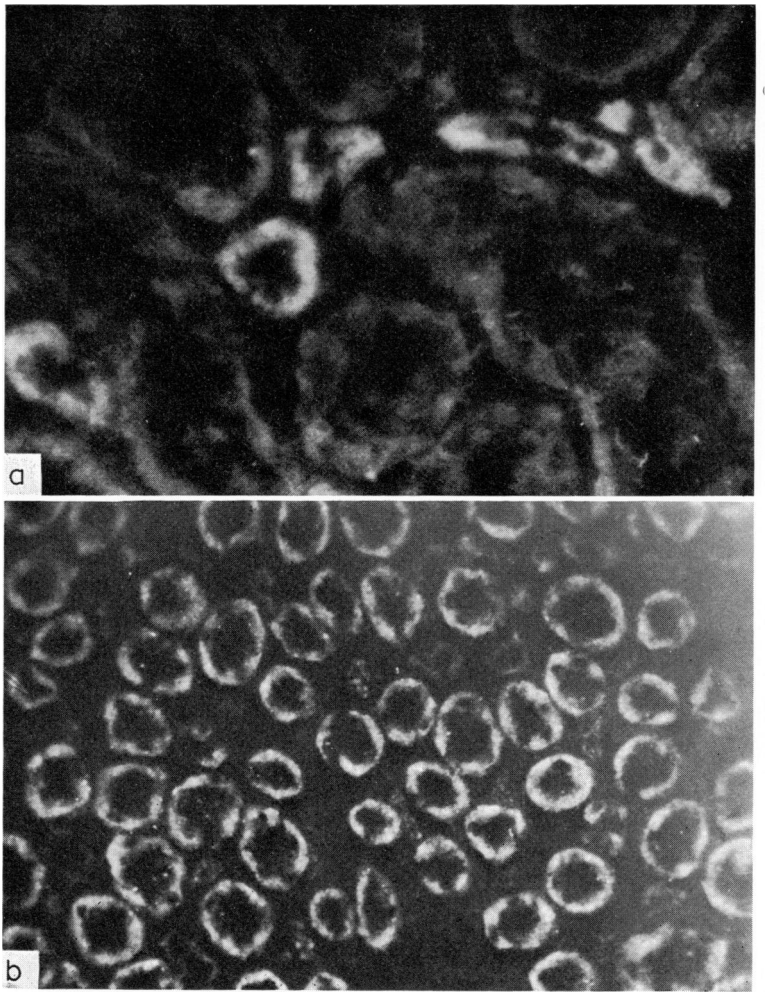

FIG. 4. (a) Rat kidney cortex, same section as in Fig. 2a, stained with rhodamine-conjugated mitochondrial antibodies. Distal tubule at centre is strongly fluorescent, proximal tubules more weakly stained. Double exposure photomicrographs showed mixture of red and green fluorescence, i.e. microsomal and mitochondrial antigens are present together in proximal tubule cells. (b) Rat kidney medulla treated as Fig. 4a showing bright fluorescence of ascending loops of Henle with mitochondrial antibodies.

mitochondria were counted in five to ten randomly selected fields in each section. Sections 3 and 11 contained between two and seven LHD tubules (mean 4·6) per field, sections 48 and 58 contained between four and fourteen (mean 7) and sections 91 and 101 showed six

to eighteen (mean 10·4) of these tubules. The medulla showed all ascending loops of Henle stained (Fig. 4b) and there was no reaction in the descending loop or in the collecting tubules. The intensity of fluorescence did not vary in LHD in different parts of the kidney. By contrast, proximal renal tubules stained more strongly in the upper cortex than in the deeper parts, with anti-mitochondrial conjugate.

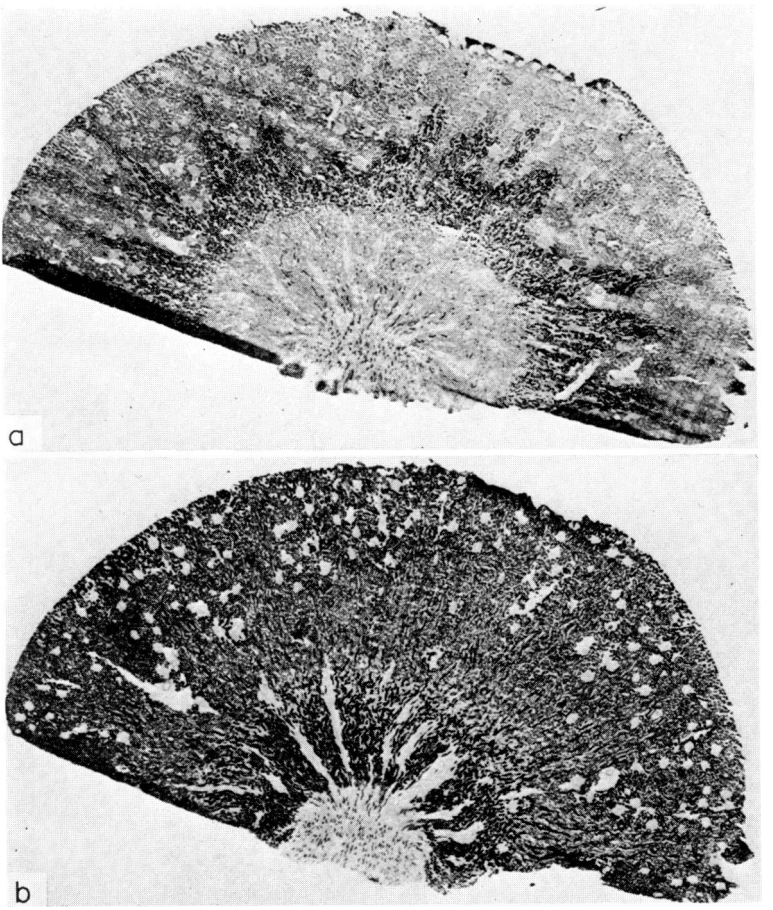

FIG. 5. Cryostat sections of rat kidney stained with peroxidase conjugates of: (a) microsomal antibodies which stained only the inner cortex containing mainly P_3 portions of proximal tubules; (b) mitochondrial antibodies which reacted with the entire cortex and medulla giving maximum staining on ascending loops of Henle and distal tubules.

With the anti-microsomal conjugate it was not possible to count tubules accurately in view of the wedged distribution of staining throughout the upper cortex. However, the same serial sections showed a gradual increase both in intensity of staining in individual proximal tubules and in the total proportion of stained tubules when going from the capsule towards the medulla, suggesting that the distal part of the proximal tubule contained the most microsomal antigen. In the deep cortex all proximal tubules were intensely fluorescent. At

the cortico-medullary junction there was an abrupt cessation of fluorescence and no stained tubules were seen below this level (Fig. 2b).

Using peroxidase conjugates of the two types of sera it was possible to take low power photographs of the entire rat kidney section. The distributions of the two antigens are shown in Figs 5a and b. These findings are summarized in Table 3. This also shows the difference in fluorescence obtained with the two antibodies on other organs.

TABLE 3. Comparison of microsomal and mitochondrial immunofluorescence

Organs and tissues	Autoantibodies	
	Microsomal	Mitochondrial
Human or rat liver	++ Fine gran.	+ Coarse gran.
Human kidney:		
Cortex	++ P	++ D>P
Medulla	Neg.	++ LH
Rat kidney:		
Outer cortex	Neg. or weak+	++D>P
Inner cortex	++ P	++ LHD
Medulla	Neg.	++ LH
Submaxillary gland:		
Ducts	±	++
Glandular cells	Neg.	±
Cardiac muscle	±	++
Striated muscle	Neg.	++ Red fibres
Pancreas	Neg.	±
Brain, adrenal	Neg.	+
Brown fat	Neg.	+++
Thyroid:		
Normal epithelium	Neg.	+
Oxyphil cells	Neg.	++
Stomach:		
Parietal cells	Neg.	++
Chief cells	Neg.	+

P = Proximal tubules. D = Distal tubules. LH = Ascending loop of Henle.

DISCUSSION

The microsomal fluorescence pattern has proved difficult to define for several reasons. The antibodies appear to be rare: over the past $1\frac{1}{2}$ years since a conscious search was made, $<0{\cdot}5\%$ of sera gave a pattern of this nature and few sera reacted to more than 1:10. The first high titre serum (JA) was studied since 1969; the liver fluorescence appeared much more intense than anything seen on kidney, thyroid or stomach and absorption studies suggested a microsomal autoantigen (Doniach *et al.*, 1971). At that time human kidney was used, and rat liver sections were only applied for ANA dilutions. Quite separately from this, we became aware of an IFL pattern on kidney, distinct from that obtained with mitochondrial anti-

bodies as seen in PBC sera, in that proximal tubules were chiefly stained instead of distal. Two of these sera (JW and CR) reacted better in CFT with rat liver mitochondria than with whole homogenate and it was wrongly concluded that proximal tubule IFL was due to a second mitochondrial antibody (Doniach & Walker, 1972). It was only when the sera reacting with liver and those reacting with proximal renal tubules were studied systematically with both organs from the rat, that the unity of these two types of reaction became clear. Quantitative CFT studies were then undertaken with purified microsomal and mitochondrial fraction, carefully checked for cross-contamination by marker enzyme tests, and these clearly showed that previous positive results with mitochondria were due to contamination with microsomes. Comparable CH_{50} curves could be constructed with these sera using less than a tenth the amount of protein/ml from the microsomal fraction than with mitochondria. In addition the kidney and liver fluorescence could be absorbed out with microsomal membranes while these did not affect the mitochondrial PBC antibodies.

Our previous misinterpretations were due to the fact that microsomal antibodies only fluoresce strongly with hepatocytes and with the third segment of the proximal tubule (P_3) (Maunsbach, 1966; Beard & Novikoff, 1969), while the first and second segments are stained irregularly with some sera and not at all with others. The reason why some microsomal sera produce a wedge-shaped fluorescence reaching the renal capsule while others stain only the inner cortex is the anatomical relationship with alternating cones of outer and inner cortex (Sternberg, Farber & Dunlap, 1956) as reflected in histochemical tests and as seen by immunoperoxidase staining in Fig. 5a.

Unless liver and the entire thickness of the rat kidney are included and one is fully conscious of inspecting each layer in the kidney, i.e. (1) outer cortex containing more first and second proximal tubule segments, (2) inner cortex which is richer in third proximal tubular segments and distal tubules, (3) outer medulla containing only loops of Henle, (4) inner medulla containing collecting tubules negative with both antibodies, it is almost impossible to distinguish microsomal from weak mitochondrial reactions.

A further difficulty is that microsomal and mitochondrial antibodies both stain hepatocyte cytoplasm. With high titre sera the patterns can be distinguished as the microsomal IFL is finely granular while mitochondrial antibodies produce a coarsely granular appearance. When the antibodies are weak the distinction is far from easy. If the sections are thicker than 5–6 μm as frequently happens when the cryostat is not in perfect working order, then it is even more difficult to say whether or not there is specific fluorescence on liver cells. Human thyroid and stomach present a more typical appearance with mitochondrial antibodies (Doniach *et al.*, 1966) and react poorly with microsomal sera. The coexistence of organ-specific reactions to these two organs in the sera of the patients makes interpretation even more difficult.

We have not yet localized this new antigen within the microsomal subfractions. Our previous report of irregular absorption of serum JA with ribosomes (Doniach *et al.*, 1971) could not be confirmed, and preliminary studies suggest that the microsomal autoantigen is in the membraneous portion of the endoplasmic reticulum vesicles. The ribosomal antibodies reported in SLE sera (Sturgill & Carpenter, 1965) are obviously quite different and we have not yet found the ACH microsomal pattern in systemic lupus. However, we have observed three sera from patients with obscure collagen disorders which gave distinctive staining patterns on liver and which appear to be anti-ribosomal (case J in Doniach *et al.*, 1971).

Complement fixation with subcellular liver fractions including microsomes were previously reported in active chronic hepatitis (Asherson, 1959; Meyer zum Buschenfelde, 1972) and in primary biliary cirrhosis (Mackay & Larkin, 1958; Deicher, Holman & Kunkel, 1960). About 25% of PBC patients fix complement significantly with purified microsomes (Doniach *et al.*, 1966) in addition to the mitochondrial CFT which is usually of higher titre. However, it was not possible to demonstrate the simultaneous presence of microsomal antibodies by immunofluorescence in these sera.

The clinical significance of the microsomal antibodies described in this paper is being evaluated and will be reported in greater detail elsewhere. It is striking that almost all the high titre sera observed so far came from patients with an active form of chronic hepatitis. These patients had none of the serological features of the other subgroups recently distinguished in this ubiquitous disease. Few of the patients had evidence of HB virus infection, nor could any be classified as 'lupoid' owing to the absence of ANA and SMA. So far there is no evidence of oxyphenisatin ingestion (Reynolds *et al.*, 1971) in the causation of the 'microsomal' cases. The age and sex distribution of ACH patients having microsomal antibodies also differed from patients with this disease showing high titre mitochondrial reactions. The latter are nearly always middle-aged females with conspicuously raised alkaline phosphatase levels, and other features reminiscent of PBC, while in the 'microsomal' cases the sex distribution was equal, and the mean age was 32 years.

The microsomal antibodies are not organ-specific and therefore probably not responsible for the initiation or maintenance of the liver lesions. Furthermore, chronic hepatitis associated with microsomal antibodies appears to be a rare disease accounting for fewer cases than the categories associated with mitochondrial or nuclear antibodies. However, it is possible that these subdivisions of chronic hepatitis may represent the expression of different aetiological factors (? viruses or drugs). If the patients could be grouped by some common features, such as serum autoantibodies of HLA tissue types (Mackay & Mossir, 1972) there might be a better chance of finding out the underlying cause of these diseases.

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REFERENCES

ASHERSON, G.L. (1959) Antibodies against nuclear and cytoplasmic cell constituents in systemic lupus erythematosus and other diseases. *Brit. J. exp. Path.* **40**, 209.

BEARD, M.E. & NOVIKOFF, A.B. (1969) Distribution of peroxisomes (microbodies) in the nephron of the rat. *J. Cell Biol.* **42**, 501.

BERG, P.A., DONIACH, DEBORAH & ROITT, I.M. (1967). Mitochondrial antibodies in primary biliary cirrhosis. 1. Localization of the antigens to mitochondrial membranes. *J. exp. Med.* **126**, 277.

BUMBALO, T.S., BELLANTI, J.A. & TERPLAN, K.L. (1962). Plasma-cell hepatitis, case report with immunologic studies. *Pediatrics*, **29**, 191.

DE DUVE, C., PRESSMAN, B.C., GIANETTO, R., WATTIAUX, R. & APPELMANS, F. (1955) Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat liver tissue. *Biochem. J.* **60**, 604.

DEICHER, H.R.G., HOLMAN, H.R. & KUNKEL, H.G. (1960) Anti-cytoplasmic factors in the sera of patients with systemic lupus erythematosus and certain other diseases. *Arthr. and Rheum.* **3**, 1.

DER VARTAINEN, D.V. & VEEGER, C. (1964) Studies on succinate dehydrogenase. 1. Spectral properties of the purified enzyme and formation of enzyme-competitive inhibitor complexes. *Biochem. Biophys. Acta*, **92**, 233.

DONIACH, D., LINDQVIST, L.H. & BERG, P.A. (1971). Non-organ-specific cytoplasmic antibodies detected by immunofluorescence. *Int. Arch. Allergy*, **41**, 501.

DONIACH, D., ROITT, I.M., WALKER, J.G. & SHERLOCK, S. (1966). Tissue antibodies in primary biliary cirrhosis, active chronic hepatitis cryptogenic cirrhosis and other liver diseases and their clinical implications. *Clin. exp. Immunol.* **1**, 237.

DONIACH, D. & WALKER, J.G. (1972) *Progress in Liver Disease* (Ed. by H. Popper and F. Schaffner), vol. IV, p. 381. Grune & Stratton, New York.

MACKAY, I.R. & GAJDUSEK, D.C. (1958) An 'autoimmune' reaction against human tissue antigens in certain acute and chronic diseases. II. Clinical correlations. *Arch. intern. Med.* **101**, 30.

MACKAY, I.R. & LARKIN, L. (1958). The significance of the presence in human serum of complement-fixing antibodies to human tissue antigens. *Aust. Ann. Med.* **7**, 251.

MACKAY, I.R. & MORRIS, P.J. (1972). Association of autoimmune active chronic hepatitis with HL-A8. *Lancet*, **ii**, 793.

MAUNSBACH, A.B. (1966) Observations on the segmentation of the proximal tubule in the rat kidney. *J. ultrastruct. Res.* **16**, 239.

MEYER ZUM BUSCHENFELDE, K.H. (1972) Immunopathogenese chronischer enzundlicher lebererkrankungen. *Ergeb. Inn. Med. Kinderhlk.* **32**, 31.

LOWRY, O.H., ROSENBROUGH, M.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265.

PETTS, V. & ROITT, I.M. (1971) Peroxidase conjugates for demonstration of tissue antibodies: evaluation of the technique. *Clin. exp. Immunol.* **9**, 407.

REYNOLDS, T.B., PETERS, R.L. & YAMADE, S. (1971) Chronic active and lupoid hepatitis caused by a laxative oxyphenisatin. *New Engl. J. Med.* **285**, 813.

ROITT, I.M. & DONIACH, D. (1969). *W.H.O. Manual for Autoimmune Serology*. Geneva.

ROITT, I.M., LING, M.R., DONIACH, D. & COUCHMAN. K.G. (1964) The cytoplasmic autoantigen of the human thyroid. I. Immunological and biochemical characteristics. *Immunology*, **7**, 375.

SHUGAR, D. (1952) The measurement of lysozyme activity and the ultraviolet inactivation of lysozyme. *Biochim. biophys. Acta*, **8**, 303.

STERNBERG, W.H., FARBER, E. & DUNLAP, C.E. (1956) Histochemical localization of specific oxidative enzymes. II. Localization of diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphoras and the succindehydrogenase system in the kidney. *J. Histochem. Cytochem.* **4**, 266.

STURGILL, B.C. & CARPENTER, R.R. (1965) Antibodies to ribosomes in systemic lupus erythematosus. *Arthr. Rheum.* **8**, 213.

TSUJI, T., OGAWE, K. & KOSAKA, K. (1965) An immunofluorescent study of circulating anti-liver antibodies in chronic liver disease. *Acta Path. Jap.* **15**, 273.

WATTIAUX, R., WIBO, M. & BAUDHUIN, P. (1963) Influences of the injection of Triton WR–1339 on the properties of rat liver lysosomes. *Lysosomes, Ciba Foundation Symposium* (Ed. by A. V. S. Renck and M. P. Cameron), p. 176. J. & A. Churchill, London.