Clin. exp. Immunol. (1980) **40**, 1–7.

REVIEW

Antigens of the human liver

I. G. McFARLANE, B. M. WOJCICKA & R. WILLIAMS *The Liver Unit, King's College Hospital and Medical School, Denmark Hill, London*

(Accepted for publication 29 October 1979)

INTRODUCTION

Early interest in the identification of human liver antigens was related to their potential value as serological markers of liver cell destruction and was confined mainly to those antigens which were demonstrably liver-specific. More recently this interest has widened to include organ-specific antigens which are possible targets of immune attack in relation to various liver diseases, as well as antigens of broader tissue distribution, since immune reactions against shared antigens might be involved in idiopathic autoimmune diseases of a systemic nature (Shulman & Centeno, 1973; McFarlane *et al.*, 1976).

Many antigenic components have now been identified both in the 'soluble' fraction of human liver (Greene, Halbert & Jequier, 1972; Meyer zum Büschenfelde & Miescher, 1972; Espinosa, 1973; Brand, 1974; Sugamura & Smith, 1976; Mihas *et al.*, 1977) and in association with intracellular organelles including microsomes (Ben-Yoseph, Shapira & Doniach, 1974) and mitochondria (Sayers & Baum, 1976; Labro *et al.*, 1978). However, the antigens of greatest current interest are undoubtedly those associated with the hepatocyte plasma membrane since these are more likely targets of damaging immune reactions *in vivo* than are wholly intracellular components.

LIVER-SPECIFIC MEMBRANE LIPOPROTEIN (LSP)

In 1972, Meyer zum Büschenfelde & Miescher reported the presence of two liver-specific antigens, LP1 and LP2, in 150,000-*g* supernatants of normal human liver. LP2 was identified as a cytoplasmic component of approximate molecular weight 190,000, while LP1—which, through common usage, has become known as LSP—was defined as a high molecular weight, species-non-specific lipoprotein associated with the plasma membrane (Meyer zum Büschenfelde & Miescher, 1972; Hopf, Meyer zum Büschenfelde & Freudenberg, 1974). Studies in our laboratories (McFarlane *et al.*, 1977) have shown that LSP is a far more complex component than was at first recognized. It has an apparent molecular weight (by gel filtration) of more than 20×10^6, contains phospholipids and triglycerides, and shows multiple protein-containing components on SDS–polyacrylamide gel electrophoresis (McFarlane *et al.*, 1977; Meyer zum Büschenfelde & Hütteroth, 1979). Moreover, Hütteroth & Meyer zum Büschenfelde (1978) have suggested that it contains at least two liver-specific determinants, one of which is species-cross-reactive and the other human-specific.

The difficulties which have been encountered in attempting to elucidate the structure of human LSP and identify and characterize its associated antigenic determinants are related to its labile nature, differences in preparative techniques employed in different laboratories and inter-species variations in responses of animals immunized with this material. In earlier studies, antisera raised against crude LSP preparations showed immunofluorescent staining of isolated rabbit hepatocytes (Hopf *et al.*, 1974; McFarlane *et al.*, 1977), Recently, Meyer zum Büschenfelde *et al.* (1979) found that antisera raised in either rabbits or sheep against more highly purified LSP (by gel filtration over

Correspondence: Dr Roger Williams, The Liver Unit, King's College Hospital and Medical School, Denmark Hill, London SE5.

0099-9104/80/0400-0001$02.00 © 1980 Blackwell Scientific Publications

Sepharose 6B) do not exhibit surface immunofluorescent staining of rabbit hepatocytes but do react against rabbit LSP on immunoelectrophoresis. Furthermore, on crossed immunoelectrophoresis against human LSP, the sheep antiserum gave two precipitin peaks while the rabbit antiserum gave only one. In our laboratories, antisera raised in guinea-pigs against human LSP, prepared in an identical manner, also failed to react against isolated hepatocytes but gave two lines on immunodiffusion against the immunizing material—one line showing complete identity with a line obtained against an extract of purified rabbit liver plasma membranes (De Kretser *et al.*, 1979).

Thus it appears that LSP contains multiple antigenic specificities, the identification of which depends to a large extent on the method of preparation and the animal species immunized. It is probably inappropriate, therefore, to describe LSP as a *lipoprotein* since this implies that it is a single entity. We suggest that it would be more accurate to describe this component as: the largest molecular species obtainable, by gel filtration over Sepharose 6B, from 100,000-*g* supernatants of fresh normal liver, comprised of a number of antigenic determinants at least one of which is liver-specific, plasma-membrane-associated and species-cross-reactive. Large size, organ specificity, plasma-membrane localization and species-cross-reactivity are the salient points of this definition and are required for maintenance of fidelity to Meyer zum Büschenfelde's original description and for ensuring continuity between studies conducted in different laboratories. It will be recognized that this is an open-ended definition which avoids the complications inevitable in attempting to relate multiple antigenic specificities to what may prove not to be a single entity. At the same time it allows for expansion of the nomenclature as new antigenic determinants become identified.

THE LMA/LM-Ag SYSTEM

Hepatocytes isolated from liver biopsy specimens from patients with HBsAg-negative chronic active hepatitis (CAH) were found by Hopf, Meyer zum Büschenfelde & Arnold (1976) to have IgG bound to their surfaces in a linear pattern as determined by staining with fluoresceinated anti-human IgG. Sera from these patients showed, by indirect immunofluorescence, a similar linear staining pattern of the surfaces of isolated rabbit hepatocytes; direct evidence, however, that the IgG found on patients' liver cells has the same specificity as the antibody which binds to rabbit hepatocytes has not yet been obtained. These findings were confirmed by Tage-Jensen *et al.* (1977) and this new autoantibody was designated: liver membrane antibody (LMA).

The characteristics of LMA are that it is directed against a species-non-specific antigen on the hepatocyte surface and is found mainly in the sera of patients with HBsAg-negative CAH. Hopf *et al.* (1976) reported that the linear pattern of fluorescent staining produced by LMA-positive sera on rabbit hepatocytes could be abolished by absorbing the sera with human LSP—implying that the antigen against which LMA is directed is a component of LSP. More recent studies in our laboratories revealed that high titres of *anti-LSP* antibodies could be detected by radioimmunoassay in the sera of patients with either HBsAg-negative or HBsAg-positive CAH (Jensen *et al.*, 1978). This finding was confirmed by Kakumu *et al.* (1979) and by Gerber *et al.* 1979) and has stimulated much discussion regarding the identity of the antigen(s) against which LMA and anti-LSP are directed. The situation is complicated further by the recent observation (De Kretser *et al.*, 1979) that sera from patients with either HBsAg-negative or HBsAg-positive CAH contain antibodies which react on immunodiffusion against a component present in both purified rabbit liver plasma membranes and human LSP.

Meyer zum Büschenfelde *et al.* (1979) have now reported the purification of an antigen (LM-Ag) by affinity chromatography, using immunoglobulin from LMA-positive, HBsAg-negative sera. These sera were selected by the criterion that the LMA could *not* be absorbed by LSP and, as a control, an affinity column coupled to immunoglobulin from LMA-negative, HBsAg-positive sera was employed. The authors found that an antigen could be extracted from both rabbit and human liver 100,000-*g* supernatants by the LMA-positive, but not by the LMA-negative column and, by absorption experiments, showed that this antigen is present on the surface of isolated rabbit hepatocytes. It is not known whether the sera used for affinity chromatography contained anti-LSP

antibodies nor, apparently, was it determined whether purified LSP would bind to the column from which LM-Ag was obtained. On the other hand, on immunoelectrophoresis, purified LM-Ag migrated in the pre-albumin region in contrast to LSP which migrates in the gammaglobulin region and this supports the authors' suggestion that LM-Ag is distinct from LSP.

On balance, the present evidence suggests that there may be at least two species-cross-reactive antigens associated with the liver cell membrane. One of these is a target for immune reactions in patients with either HBsAg-negative or HBsAg-positive CAH, stimulates antibody responses when injected into animals and is present in purified LSP. The other, LM-Ag, is a target for antibodies found mainly in the sera of patients with HBsAg-negative CAH and either does not readily produce antibody responses in animals or is not a component of purified LSP (although it may have been present in the crude LSP preparations used in earlier studies). The relationship between these antigens and the murine cell membrane liver-specific antigens studied by Behrens & Paronetto (1978) is as yet unknown.

BILIARY TRACT ANTIGENS

Because of the technical problem of dissecting workable amounts of bile duct and bile ductule tissue away from the surrounding hepatic parenchyma, investigations of biliary antigens have been confined to studies on bile—on the assumption that components derived from the biliary tract may be shed or secreted into the lumens of the bile ducts. Hardwicke *et al.* (1964) showed that although the major part of the protein in bile is comprised of albumin and globulin, a significant proportion can not be accounted for in terms of plasma proteins. Subsequently, Yoon, Shim & Kil (1966) identified, in normal human bile, three 'bile-specific' components (distinct from plasma proteins) which were designated biliprealbumin, biliproalbumin and α_1-biliprotein on the basis of electrophoretic mobility. Similar findings were reported by Englert, Wales & Straight (1970) and by Espinosa (1976) who identified a total of four such 'bile-specific' proteins.

In our laboratories, the antigenic composition of a protein fraction of normal human gallbladder bile, against which *in vitro* cellular immune responses were demonstrable in patients with primary biliary cirrhosis (Eddleston *et al.*, 1973; McFarlane *et al.*, 1979), has been investigated. This bile protein fraction, obtained by ammonium sulphate fractionation and gel filtration over Sepharose 6B, was found to contain three distinct antigens. Antigens I and II are glycoproteins with approximate molecular weights of 170,000 and 260,000 respectively, while antigen III is a lipoprotein with an approximate molecular weight of 790,000 as determined by gel filtration (Wojcicka, 1979). Immunofluorescence studies revealed that antigen II is associated with the canalicular portion of the hepatocyte membrane while antigen III appears to be derived from the membranes of intrahepatic bile duct epithelial cells. All three antigens exhibit broad, but distinct patterns of cross-reactions with other tissues and in this respect are similar to the antigens described by Espinosa (1976). The relationship between these antigens and a biliary glycoprotein (BGP I), which cross-reacts with carcinoembryonic antigen (Svenberg, Hammarström & Zeromski, 1979) and which appears to be associated with the bile canaliculi, is unknown.

NEOANTIGENS

In recent years several abnormal antigenic liver cell constituents have been identified in association with certain diseases of the liver. These neoantigens are immunogenic components which are not found in histologically normal tissue but which appear in diseased liver as a result of some exogenous or endogenous stimulus and include substances such as hyalin and abnormal forms of ferritin. The immunogenic properties of hyalin have been studied by Leevy and his co-workers who have described both cellular (Zetterman, Luisada-Opper & Leevy, 1976; Kakumu & Leevy, 1977) and humoral (Kanagasundaram *et al.*, 1977) immune responses to this material in patients with alcoholic liver disease, while abnormal ferritins, physicochemically and immunologically distinguishable from normal liver ferritin, have been identified in rat and human hepatocellular carcinoma (Alpert, Coston & Drysdale, 1973; Bullock, Bomford & Williams, 1979).

Of particular interest are novel antigens which appear on the hepatocyte surface. Early studies

of such neoantigens were concerned with the mechanism of liver cell destruction in acute type B viral hepatitis. The hepatitis B virus is thought not to be directly cytopathic since its presence can be detected in hepatocytes from infected subjects who have no evidence of liver disease (Hadziyannis *et al.*, 1972; Krawczynski *et al.*, 1972). Rather, hepatocellular destruction in acute hepatitis appears to be related to a host immune response against viral determinants and extensive studies have now established that hepatitis B viral antigens, or virus-altered host antigens, are expressed on the surfaces of hepatocytes from infected patients (Alberti *et al.*, 1976; Ray *et al.*, 1976; Huang & Neurath, 1979).

Similar mechanisms may be involved in drug hypersensitivity reactions affecting the liver. A growing list of drugs, including the anaesthetic halothane and the laxative oxyphenisatin, have been implicated in acute and chronic hepatitis-like illnesses in a small percentage of patients (Zimmerman 1978a, 1978b). Neither halothane nor many of the other agents which give rise to these inflammatory hepatic lesions, are predictably hepatotoxic and the accompanying clinical and histological features are suggestive of hypersensitivity reactions against the drug, its metabolites, or host components altered by the drug (Klatskin, 1975; Klatskin & Smith, 1975). If host immune responses against drug haptens or drug-altered host antigens are involved, it should be possible to demonstrate the presence of such neoantigens on the surface of hepatocytes. Indirect evidence for the involvement of halothane-altered liver cell surface constituents has been derived recently from studies by Vergani *et al.* (1978) who found that sera from patients with halothane hepatitis contained antibodies which reacted against surface components of liver cells obtained from rabbits treated with halothane.

The processes which lead to the expression of drug- or virus-related neoantigens on the hepatocyte surface are not known. Drugs could produce such alterations by direct action on the plasma membrane but such a mechanism does not seem to operate in the case of the hepatitis B virus which appears to make its presence known from within the cell. A more likely hypothesis is that these neoantigens arrive on the cell surface during the normal process of plasma membrane turnover.

Current concepts of plasma membrane biogenesis fall, broadly, into two schools: (a) the membrane flow concept, which postulates a continuity between the endoplasmic reticulum (ER) and the plasma membrane, and (b) the direct synthesis concept by which membrane constituents are inserted directly from the cytosol. The evidence in support of each school has been reviewed by Morré, Kartenbeck & Franke (1979) who propose an intermediate concept in which membrane flow (including membrane retrieval and re-utilization) accounts for the bulk of the plasma membrane, with these endomembrane-derived elements being modified by additions and deletions during transit or after insertion.

The evidence in favour of vectorial migration of membrane components from the ER to the plasma membrane is now considerable and points to a mechanism whereby membranes of secretory vesicles such as the Golgi apparatus, formed by 'budding' from the ER (Jamieson & Palade, 1967; Claude, 1970), interact and fuse with the plasma membrane during exocytosis. In this way, new membrane segments may carry different specificities into the plasma membrane (Masur *et al.*, 1971). Studies on viruses have established that such a flow mechanism can account for the appearance of viral determinants on the cell surface (Rifkin & Quigley, 1974; Ehrnst & Sundqvist, 1976; Rothman & Lenard, 1977) and it is interesting that electron microscopical studies have shown alterations in, and association of viral components with, the ER of hepatitis B virus-infected liver cells (Huang & Groh, 1973; Gudat *et al.*, 1975).

A similar mechanism could operate in the production of drug-related cell surface neoantigens. It has been shown that the metabolism of many drugs (including halothane) by the liver involves their conversion to reactive intermediates which bind covalently to the ER (Mitchell & Jollows, 1975; Uehleke & Werner, 1975) and it is conceivable that such endomembrane-bound drug haptens (or drug-altered host components) could be transported to the plasma membrane and expressed on the cell surface as neoantigens.

CONCLUSION

To date, little attention has been devoted to possible functional characteristics of human liver cell

antigens. A number of intracellular immunogenic components have been identified as enzymes (Greene *et al.*, 1972; Rose, Milisauskas & Sampson, 1975), others may be carrier proteins associated with the various metabolic functions of the hepatocyte or export proteins in incomplete or zymogenic forms. Even where antigens have been highly purified and characterized, little is known about the number or nature of the antigenic sites (epitopes) on these molecules. With the plasma-membrane-associated antigens such information would be of considerable interdisciplinary interest since some epitopes may prove to be receptor sites for vitamins, hormones and various metabolites involved in functions unique to the liver. Progress in this area should help to solve many of the questions which have been raised in recent years concerning the mechanisms of liver damage in acute and chronic diseases of the liver.

REFERENCES

ALBERTI, A., REALDI, G., TREMOLADA, F. & SPINA, G.P. (1976) Liver cell localization of hepatitis B antigen and of immunoglobulins in acute and chronic hepatitis and in liver cirrhosis. *Clin. exp. Immunol.* **25**, 396.

ALPERT, E., COSTON, R.L. & DRYSDALE, J.W. (1973) Carcinofetal human liver ferritins. *Nature*, **242**, 194.

BEHRENS, U.J. & PARONETTO, F. (1978) Liver-specific and shared cell membrane antigens. Studies by light- and electron microscopy. *Immunology*, **35**, 289.

BEN-YOSEPH, Y., SHAPIRA, E. & DONIACH, D. (1974) Further purification of the mitochondrial inner membrane autoantigen reacting with primary biliary cirrhosis sera. *Immunology*, **26**, 311.

BRAND, S. (1974) Some properties of the liver-specific delta autoantigen of the rat. *Pathol. Microbiol.* **40**, 326.

BULLOCK, S., BOMFORD, A. & WILLIAMS, R. (1979) A biochemical comparison between ferritins from normal human liver and hepatocellular carcinoma. *Biochem. J.* (In press.)

CLAUDE, A. (1970) Growth and differentiation of cytoplasmic membranes in the course of lipoprotein granule synthesis in the hepatic cell. I. Elaboration of elements of the Golgi complex. *J. Cell Biol.* **47**, 745.

DE KRETSER, T.A., MCFARLANE, I.G., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1979) A species-non-specific liver plasma membrane antigen and its involvement in chronic active hepatitis. *Biochem. J.* (In press.)

EDDLESTON, A.L.W.F., MCFARLANE, I.G., MITCHELL, C.G., REED, W.D. & WILLIAMS, R. (1973) Cell mediated immune response in primary biliary cirrhosis to a protein fraction form human bile. *Br. med. J.* **iv**, 274.

EHRNST, A. & SUNDQVIST, K.G. (1976) The mechanisms of appearence of viral glycoproteins at the cell surface membrane. *Exp. Cell Biol.* **44**, 198.

ENGLERT, E., WALES, E.E. & STRAIGHT, R.C. (1970) The proteins of human gallbladder bile with and without gallstones. *Clin. Chim. Acta*, **29**, 319.

ESPINOSA, E. (1973) Circulating tissue antigens. II. Studies on an organ-specific antigen of human liver. *Lab. Invest.* **29**, 556.

ESPINOSA, E. (1976) Circulating tissue antigens. III. Identification and characterization of antigens of limited and of wide body distribution in human gallbladder bile. Presence in serum of patients with hepatitis. *Clin. exp. Immunol.* **25**, 410.

GERBER, M.A., LEBWOHL, N., THUNG, S.N., BODENHEIMER, H. & SCHAFFNER, F. (1979) Antibodies to liver-specific membrane protein in liver and kidney disease. *Gastroenterology*, **76**, 1281 (abstract).

GREENE, E.L., HALBERT, S.P. & JEQUIER, S. (1972) Analysis of the tissue constituents and enzymes of human liver by crossed immunoelectrophoresis—comparison of normal and cystic fibrosis liver. *Int. Arch. Allergy appl. Immunol.* **42**, 751.

GUDAT, F., BIANCHI, L., SONNABEND, W., THIEL, G., AENISHAENSLIN, W. & STALDER, G.A. (1975) Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. *Lab. Invest.* **32**, 1.

HARDWICKE, J., RANKIN, J.G., BAKER, K.J. & PREISIG, R. (1964) The loss of protein in human and canine hepatic bile. *Clin. Sci.* **26**, 509.

HADZIYANNIS, S., HOUSSOUROS, A., VISSOULIS, C. & AFROUDAKIS, A. (1972) Cytoplasmic localization of Australia antigen in the liver. *Lancet*, **i**, 976.

HOPF, U., MEYER ZUM BÜSCHENFELDE, K.H. & ARNOLD, W. (1976) Detection of a liver membrane autoantibody in HBsAg-negative chronic active hepatitis. *N. Engl. J. Med.* **294**, 578.

HOPF, U., MEYER ZUM BÜSCHENFELDE, K.K. & FREUDENBERG, J. (1974) Liver-specific antigens of different species. II. Localization of a membrane antigen at cell surface of isolated hepatocytes. *Clin. exp. Immunol.* **16**, 117.

HUANG, S.-N. & GROH, C. (1973) Immunoagglutination electron microscope study on virus-like particles and Australia antigen in liver tissue. *Lab. Invest.* **29**, 353.

HUANG, S.-Z. & NEURATH, A.R. (1979) Immunohistologic demonstration of hepatitis B viral antigens in liver with reference to its significance in liver injury. *Lab. Invest.* **40**, 1.

HÜTTEROTH, T.H. & MEYER ZUM BÜSCHENFELDE, K.H. (1978) Clinical relevance of the liver-specific lipoprotein (LSP). *Acta Hepatogastroenterol.* **25**, 243.

JAMIESON, J.D. & PALADE, G.E. (1967) Intracellular transport of secretory proteins in the pancreatic exocrine cell. II. Transport to condensing vacuoles and zymogen granules. *J. Cell Biol.* **34**, 577.

JENSEN, D.M., MCFARLANE, I.G., PORTMANN, B.S., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1978)

Detection of antibodies directed against a liver-specific membrane lipoprotein in patients with acute and chronic active hepatitis. *N. Engl. J. Med.* **299,** 1.

KAKUMU, S., ARAKAWA, Y., GOJI, H., KASHIO, T. & YATA, K. (1979) Occurrence and significance of antibody to liver-specific membrane lipoprotein by doube-antibody immunoprecipitation method in sera of patients with acute and chronic liver diseases. *Gastroenterology,* **76,** 665.

KAKUMU, S. & LEEVY, C.M. (1977) Lymphocyte cytotoxicity in alcoholic hepatitis. *Gastroenterology,* **72,** 594.

KANAGASUNDARAM, N., KAKUMU, S., CHEN, T. & LEEVY, C.M. (1977) Alcoholic hyalin antigen (AHAg) and antibody (AHAb) in alcoholic hepatitis. *Gastroenterology,* **73,** 1368.

KLATSKIN, G. (1975) Toxic and drug-induced hepatitis. *Diseases of the Liver* 5th edn. (ed. by L. Schiff), p. 604, J.P. Lippincott, Philadelphia.

KLATSKIN, G. & SMITH, D.P. (1975) Halothane-induced hepatitis. *Drugs and the Liver* (ed. by W. Gerok and K. Sickinger), p. 289. F.K. Schattauer-Verlag, Stuttgart.

KRAWCZYNSKI, K., NAZAREWICZ, T., BRZOSKO, W. & NOWOSLAWSKI, A. (1972) Cellular localization of hepatitis-associated antigen in the liver of patients with different forms of hepatitis. *J. infect. Dis.* **126,** 372.

LABRO, M.T., ANDRIEU, M.C., WEBER, M. & HOMBERG, J.C. (1978) A new pattern of non-organ- and non-species-specific anti-organelle antibody detected by immunofluorescence: the mitochondrial antibody number 5. *Clin. exp. Immunol.* **31,** 357.

MASUR, S.K., HOLTZMAN, E., SCHWARTZ, I.L. & WALTER, R. (1971) Correlation between pinocytosis and hydro-osmosis induced by neurohypophyseal hormones and mediated by adenosine 3′5′-cyclic monophosphate. *J. Cell Biol.* **49,** 582.

MCFARLANE, I.G., WOJCICKA, B.M., TSANTOULAS, D.C., FUNK, C., PORTMANN, B., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1976) Cellular immune responses to salivary antigens in autoimmune liver with Sicca syndrome. *Clin. exp. Immunol.* **25,** 389.

MCFARLANE, I.G., WOJCICKA, B.M., TSANTOULAS, D.C., PORTMANN, B.C., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1979) Leucocyte migration inhibition in response to biliary antigens in primary biliary cirrhosis, sclerosing cholangitis and other chronic liver diseases. *Gastroenterology,* **76,** 1333.

MCFARLANE, I.G., WOJCICKA, B.M., ZUCKER, G.M., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1977) Purification and characterization of human liver-specific membrane lipoprotein (LSP). *Clin. exp. Immunol.* **27,** 381.

MEYER ZUM BÜSCHENFELDE, K.H. & HÜTTEROTH, T.H. (1979) Autoantibodies against liver membrane antigens in chronic active liver diseases. *Immune Reactions in Liver Disease* (ed. by A.L.W.F. Eddleston, J.C.P. Weber and R. Williams), p. 12. Pitman Medical, Tunbridge Wells.

MEYER ZUM BÜSCHENFELDE, K.H., MANNS, M., HÜTTEROTH, T.H., HOPF, U. & ARNOLD, W. (1979) LM-Ag and LSP—two different target antigens involved in the immunopathogenesis of chronic active hepatitis? *Clin. exp. Immunol.* **37,** 205.

MEYER ZUM BÜSCHENFELDE, K.H. & MIESCHER, P.A. (1972) Liver-specific antigens: purification and characterization. *Clin. exp. Immunol.* **10,** 89.

MIHAS, A.A., SACCOMANI, G., TOMANA, M. & SPENNEY, J.G. (1977) Isolation and characterization of a liver-specific antigen (F-Ag) from human liver. *Immunochemistry,* **14,** 727.

MITCHELL, J.R. & JOLLOWS, D.J. (1975) Metabolic activation of drugs to toxic substances. *Gastroenterology,* **68,** 392.

MORRÉ, D.J., KARTENBECK, J. FRANKE, W.W. (1979) Membrane flow and interconversions among endomembranes. *Biochim. Biophys. Acta,* **559,** 71.

RAY, M.B., DESMET, V.J., FEVERY, J., DE GROOTE, J., BRADBURNE, A.F. & DESMYTER, J. (1976) Distribution patterns of hepatitis B surface antigen (HBsAg) in the liver of hepatitis patients. *J. clin. Pathol.* **29,** 94.

RIFKIN, D.B. & QUIGLEY, J.P. (1974) Virus-induced modification of cellular membranes related to viral structure. *Annu. Rev. Microbiol.* **28,** 325.

ROSE, N.R., MILISAUSKAS, V. & SAMPSON, H.A. (1975) Species-specific tissue antigens. III. Immunological relationships of enzymic antigens in various species. *Clin. exp. Immunol.* **20,** 359.

ROTHMAN, R.E. & LENARD, J. (1977) The nature of membrane asymmetry provides clues to the puzzle of how membranes are assembled. *Science,* 195, 743.

SAYERS, T. & BAUM, H. (1976) Possible cross-reactivity of human antimitochondrial antibodies with membrane vesicles of *Paracoccus denitrificans. Biochem. Soc. Trans.* **4,** 138.

SHULMAN, S. & CENTENO, E.R. (1973) Isolation and characterization of a rabbit non-tissue-specific autoantigen which is shared by kidney, heart adrenal and liver. *Immunology,* **24,** 925.

SUGAMURA, K. & SMITH, J.B. (1976) Purification and characterization of human liver-specific F antigen. *Clin. exp. Immunol.* **26,** 28.

SVENBERG, T., HAMMARSTRÖM, S. & ZEROMSKI, J. (1979) Immunofluorescence studies on the occurrence and localization of the CEA-related biliary glycoprotein I (BGP I) in normal human gastrointestinal tissues. *Clin. exp. Immunol.* **36,** 436.

TAGE-JENSEN, U., ARNOLD, W., DIETRICHSON, O., HARDT, F., HOPF, U., MEYER ZUM BÜSCHENFELDE, K.H. & NIELSEN, J.O. (1977) Liver-cell membrane autoantibody specific for inflammatory liver disease. *Br. med. J.* **i,** 206.

UEHLEKE, H. & WERNER, TH. (1975) A comparative study on the irreversible binding of labelled halothane, trichlorofluoromethane, chloroform and carbon tetrachloride to hepatic protein and lipids *in vitro* and *in vivo. Arch. Toxicol. (Berl.),* **34,** 289.

VERGANI, D., MIELI-VERGANI, G., ALBERTI, A., EDDLESTON, A.L.W.F., DAVIS, M. & WILLIAMS, R. (1978) Demonstration of a circulating antibody to halothane altered hepatocytes in patients with hepatitis. *Gut,* **19,** A994 (abstract).

WOJCICKA, B. (1979) Separation and characterisation of bile antigens. *Immune Reactions in Liver Disease* (ed. by A.L.W.F. Eddleston, J.C.P. Weber and R. Williams), p. 154. Pitman Medical, Tunbridge, Wells.

YOON, D.S., SHIM, B.S. & KIL, T.S. (1966) Bile-speci-

fic protein components in human hepatic bile. *J. Lab. clin. Med.* **67,** 640.

ZETTERMAN, R.K., LUISADA-OPPER, A. & LEEVY, C.M. (1976) Alcoholic hepatitis: cell-mediated immunologic responses to alcoholic hyalin. *Gastroenterology,* **70,** 382.

ZIMMERMANN, H.J. (1978a) *Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver,* Chapter 16. Appleton-Century-Crofts, New York.

ZIMMERMAN, H.J. (1978b) *Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver,* Chapter 17. Appleton-Century-Crofts, New York.