The decision when to abandon treatment is difficult.

At the initial phase of resuscitation normal tests for death should not be accepted. However, refractory asystolic arrest and a rapid fall in body temperature unresponsive to rewarming are reasonable criteria of death.

Conclusion

Any person who receives a lightning injury and does not regain consciousness within one minute should receive external cardiac massage and mouth-to-mouth resuscitation until ventilation and peripheral perfusion is adequate. At the time of lightning shock generalized paralysis may develop which generally disappears after a few minutes, and complete restoration seldom requires more than 24 hours (Dannhorn, 1937). Occasionally paralysis may persist as a result of cerebral oedema or a prolonged period of hypoxia (Morikawa and Steichen, 1960).

Fixed dilated pupils are well recognized after lightning stroke and should not be used as an indicator of cerebral death. E.E.G. records have not been taken from many patients with lightning strike (Gathier, 1960) and therefore should be interpreted in association with the clinical findings.

In the light of present medical experience we feel it reasonable to ensure adequate ventilation and maintain metabolic and fluid balance until recovery or death.

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PRELIMINARY COMMUNICATIONS

Cell-mediated Immune Response in Primary Biliary Cirrhosis to a Protein Fraction from Human Bile

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Summary

Cell-mediated immune responses to a protein fraction of human bile have been demonstrated, using the leucocyte migration test, in eight out of 10 patients with primary biliary cirrhosis but in only three out of nine with active chronic hepatitis. In the latter condition sensitization to a liver-specific hepatocellular antigen was found more frequently (five out of nine patients) than in primary biliary cirrhosis (two out of 10). These results, as well as the granuloma formation observed histologically, suggest that the initial bile duct lesion in primary biliary cirrhosis may be associated with a cell-mediated response to antigens—perhaps derived from bile duct epithelial cells—which may be normal constituents of hepatic bile.

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Introduction

Little is known about the pathogenesis of primary biliary cirrhosis, though because of the presence of autoantibodies in the serum it is often thought to be autoimmune. In previous studies we have found evidence of cell-mediated immunity to a liver-specific lipoprotein both in this condition and in active chronic hepatitis which may also be due to an autoimmune reaction (Miller *et al.*, 1972). Immunofluorescent studies have shown that this lipoprotein is a normal constituent of the hepatocyte cell membrane (Hopf *et al.*, 1973), and sensitization to it could be of importance in the production of hepatocyte damage, which, though more characteristic of active chronic hepatitis, is also present to a varying degree in primary biliary cirrhosis. The earliest lesions in primary biliary cirrhosis involve the interlobular bile ducts (Scheuer, 1968) and could result from a reaction directed against bile duct antigens. This has proved difficult to demonstrate, mainly because of the technical problems involved with obtaining in man bile duct cells separate from hepatocytes. The possibility that lining mucosal cells could be shed into the lumen with release of antigenic material prompted the present study in which human bile was examined for an immunologically active protein fraction. The leucocyte migration test was used to detect cell-mediated immune responses to this bile protein fraction in patients with classical features of either primary biliary cirrhosis or active chronic hepatitis and in nine healthy volunteers. The results have been compared with those obtained in the same subjects when using the hepatocyte lipoprotein as antigen.

Methods

Preparation of Bile Antigen.—Hepatic bile was collected by T-tube drainage from two patients during the first 24 hours after a cholecystectomy for cholelithiasis. Each bile sample was dialysed for three days against phosphate-buffered saline, pH 7·2, and the proteins were precipitated with ammonium

sulphate. Initial studies indicated that the antigenic material was precipitated between 30% and 50% saturation. The precipitates were redissolved in phosphate-buffered saline and subjected to gel filtration on Sephadex G-50 (equilibrated with phosphate-buffered saline). The first peak eluted from the column was concentrated by pressure dialysis.

Preparation of Liver-specific Lipoprotein.—Supernatant from a homogenate of human liver obtained shortly after death and centrifuged at 150,000 *g* was subjected to sequential gel filtration by the method previously described (Miller *et al.*, 1972), except for the addition of 1 mM ethylenediaminetetra-acetic acid (EDTA) to the TRIS-HCl buffer. The EDTA stabilizes the lipoprotein, which can now be kept in solution for up to nine months at 4°C without significant loss of material or antigenicity.

Leucocyte Migration Test.—Peripheral blood leucocytes are allowed to migrate out of capillary tubes for 20 hours at 37°C into chambers containing tissue culture medium and the area of migration in the presence of an antigen is compared with that found in control chambers (Mitchell *et al.*, 1972). The liver-specific lipoprotein was used at a concentration of 50 μg protein/ml of medium and, as the EDTA in the buffer system produces slight non-specific inhibition of migration, an equivalent volume of buffer was added to the control chamber. Measurements of migration index in the nine normal subjects gave a mean value of 0·90 with a lower limit of normal (2 S.D. below the mean) of 0·74. The two bile protein preparations were used at concentrations of 100 and 400 μg protein/ml respectively—just below those which produced non-specific inhibition of migration. Measurements in the normal subjects gave a mean value for the migration index of 1·07 with a lower limit of normal of 0·83. The phosphate buffered saline in which the bile proteins were dissolved was shown not to influence migration.

Results

With bile protein as antigen, inhibition of migration was found in eight out of 10 patients with primary biliary cirrhosis (fig. 1). Statistical comparison (by Fisher's exact text) between this group and the control group showed a significant difference ($P < 0·1$). Staging of the disease by histological criteria (Scheuer, 1968) showed that in three patients it was relatively early (stage 2). In these patients the migration index was abnormal, the normal values being found in two of the remaining seven, all of whom were thought to have advanced (stage 4) disease. No correlation could be detected between the degree of migration inhibition and the serum bilirubin, aspartate aminotransferase, or alkaline phosphatase levels, or the titre of antimitochrondrial antibody in the serum.

In active chronic hepatitis three of the nine patients tested with bile protein as antigenic material showed inhibition of migration. This result was not significantly different from either the controls or the group with primary biliary cirrhosis. One of the abnormal results was in a patient who had severe cholestasis with pruritus and a considerably raised serum alkaline phosphatase level. No relation could be detected with serum antimitochrondrial antibody which was present in three patients, all of whom had normal migration indices. There did not appear, however, to be a difference in the sex ratio, three of the four males in the series showing inhibition of migration while the five females all gave normal values.

When the liver-specific lipoprotein was used as antigen in the leucocyte migration test in these same patients a quite different pattern was found to that obtained with the bile protein (fig. 2). Inhibition of migration was observed in only two of the 10 patients with primary biliary cirrhosis ($P > 0·10$ compared with controls) whereas it was found in five of the

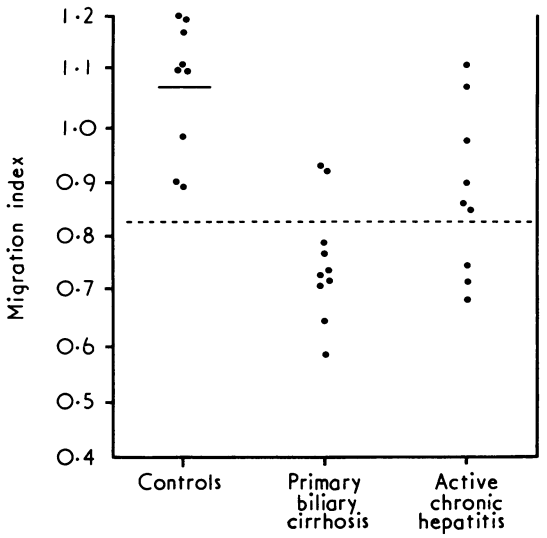

FIG. 1—Results of leucocyte migration test with bile protein as antigen. Lower limit of normal (- -) is 2 S.D. below mean (—) of control group.

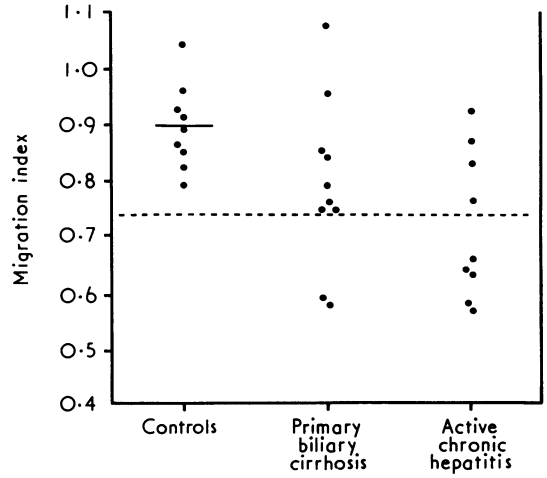

FIG. 2—Results of leucocyte migration test with liver-specific lipoprotein as antigen. Lower limit of normal (- -) is 2 S.D. below mean (—) of control group.

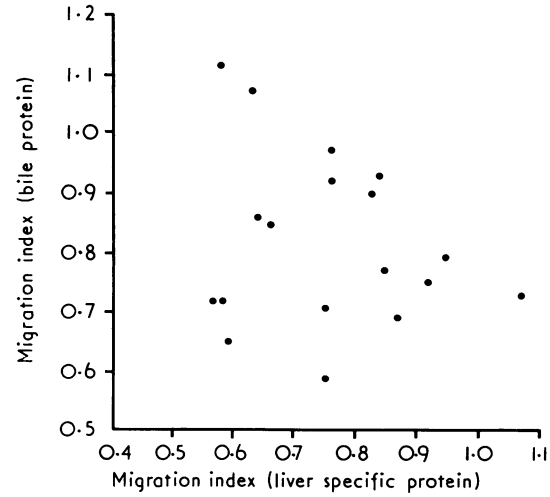

FIG. 3—Migration index obtained with bile protein as antigen compared with result using liver-specific lipoprotein for each patient.

nine with active chronic hepatitis ($P<0.05$). There was no correlation when the values obtained with lipoprotein were compared with those using bile protein on the same sample of blood (fig. 3).

Discussion

Though the leucocyte migration test developed by Søborg and Bendixen (1967) was for some time regarded as an unreliable test of cell-mediated immunity, recent studies have verified its value as an in-vitro correlate of delayed hypersensitivity (Rosenberg and David, 1970; Federlin *et al.*, 1971; Mitchell *et al.*, 1972). In the context of liver disease the test has been used to demonstrate cell-mediated immune responses in autoimmune liver disease (Miller *et al.*, 1972; Smith *et al.*, 1972), hepatitis B infection (Dudley et al., 1972), and during rejection episodes after liver transplantation (Eddleston *et al.*, 1971). It is relatively insensitive and the most consistent results are found when purified antigens are used, though our latest results with the liver-specific lipoprotein (Miller *et al.*, 1972) are similar to those obtained initially with a liver homogenate (Smith *et al.*, 1972).

Thus the present findings may reasonably be taken as evidence of a cell-mediated immune response to bile antigens in these patients. Clearly it will be important to purify the antigens in the protein fractions used and to determine their exact source. Other workers have identified two bile-specific proteins in human hepatic bile (Yoon *et al.*, 1966) and it is of some interest that one of these is found in the same fraction (after Sephadex gel filtration) as in our preparation. A bile-specific antigen has also been found in dogs (Hardwicke *et al.*, 1964).

Other evidence suggesting that a cell-mediated immune reaction may play an important part in the pathogenesis of primary biliary cirrhosis includes the granulomata often seen around damaged bile ducts in the early stage of the disease. In certain situations, such as infections with intracellular pathogens, granulomata are indicative of a vigorous cell-mediated reaction. The ability of lymphocytes to activate macrophages has been described recently, and it has also been shown that such activated macrophages can mature into epithelioid cells (Dannenberg *et al.*, 1968). The results of Fox *et al.* (1969) showing impaired immune responsiveness in primary biliary cirrhosis were obtained in the later stages of the disease, and do not seem to be implicated in pathogenesis. The antimitochondrial antibody found so often in the serum of these patients is not organ-specific and is unlikely to be important in the production of the bile duct lesion. In a few patients deposits of immunoglobulin and complement have been detected by immunofluorescence around bile duct epithelial cells (Paronetto and Popper, 1971), suggesting the presence of a specific antibody reacting with bile duct antigens. It is of course possible that both humoral and cellular responses are involved as has been found in autoimmune disorders induced experimentally in animals (Brown *et al.*, 1967).

In the later stages of primary biliary cirrhosis there is extension of the inflammatory reaction into the hepatic parenchyma and the histological appearances can resemble those found in active chronic hepatitis. Though the finding of sensitization to the hepatocyte lipoprotein was not closely related to the histological staging of the disease, the latter is relatively crude and such sensitization may be of importance in the pathogenesis of parenchymal damage. Finally, the present results show that cell-mediated immune responses, as with histological changes and autoantibodies, show some overlap between primary biliary cirrhosis and active chronic hepatitis. Clinically, however, these diseases are usually distinct and whether the two conditions are but parts of a spectrum of autoimmune liver disease (Doniach, 1970) will not be established until the factors triggering these immunological responses are identified.

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