at 12 noon leading to a rise from 0.85 $\mu\text{g/ml}$ at 11.15 a.m. to 4.32 $\mu\text{g/ml}$ at 12.15 p.m. The second unusual feature is the high peak concentration of levodopa, 4.32 $\mu\text{g/ml}$. A third point of contrast is the prolonged plasma half life of levodopa in our patient, 1.57 hours compared with 1.08 hours.

The mean scores for dysphonia, confusion, and facial hypokinesia are also indicated in the graph. It is evident that high plasma concentrations correlate with maximal deficit in all clinical features.

After these investigations levodopa therapy was stopped, and orphenadrine (50 mg thrice daily) was given. It is notable that the episodes of dysphonia and confusion cleared completely over four weeks without any general deterioration in her Parkinsonism.

Discussion

The "on-off" phenomenon has become recognized as a problem of increasing importance over the last year. Its relatively recent emergence can be explained in terms of prolonged periods of therapy usually completed before its appearance. Attacks are particularly distressing when, as often occurs, they develop in patients who had done well on levodopa. No satisfactory approach to their management has been reported.

The simplest explanation for a dramatic increase in hypokinesia in patients receiving levodopa would be inadequate intake or absorption of the drug. The findings reported here indicate that it is more likely that the attacks represent a toxic reaction to levodopa or one of its metabolites. This view would be consonant with the reported association of these hypokinetic episodes with certain known adverse reactions to levodopa such as confusion and hyperkinesia, which are also accompanied by high plasma concentrations of levodopa. Furthermore, the higher prevalence of "on-off" phenomena in patients who have been on treatment with high doses for prolonged periods is in accord with the view that a build-up of levodopa or one of its metabolites is responsible.

While the mechanism of levodopa toxicity in these episodes is not known, Hornykiewicz (1973) has made a suggestion, entirely compatible with our observations, that a metabolite of levodopa is acting as a "partial agonist" or "false transmitter" in the central nervous system.

One feature of interest in the patient studied here was the

extremely rapid absorption of levodopa. As the plasma half life was prolonged, it is possible that either metabolism was impaired or distribution was abnormal. In patients who develop "on-off" attacks, therefore, unusual pharmacokinetics may prove to be a contributing factor.

Having obtained this evidence that "on-off" phenomena are related to excessive plasma concentrations of levodopa, certain practical implications should be considered. When starting patients on levodopa it may be unwise to increase the dose to maximum tolerated levels in all cases. Barbeau (1972) also concluded from clinical observations that "on-off" attacks represent levodopa toxicity, and he has suggested that an arbitrary upper dose limit around 4.0 g a day should be set for routine therapy until further evidence is available.

In addition to preventing attacks there is the problem of managing patients in whom these episodes are established. It is evident that unless the excessive peaks of plasma levodopa can be reduced by more frequent administration of smaller doses the total daily intake of levodopa should be decreased. The addition of peripheral decarboxylase leads to less extreme fluctuations in the plasma concentration of levodopa through the day, but mean plasma concentrations tend to be rather higher so it is not possible to predict how "on-off" phenomena will be affected.

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References

- Barbeau, A., Gillo-Joffroy, L., and Mars, H. (1971). *Clinical Pharmacology and Therapeutics*, 12, 353.
- Barbeau, A. (1972). In *Proceedings of the 4th International Symposium on Parkinson's Disease*, ed. J. Siegfried. Bern, Huber.
- Calne, D. B., et al. (1971). *British Medical Journal*, 3, 729.
- Cotzias, G. C., Papavasiliou, P. S., and Gellene, R. (1969). *New England Journal of Medicine*, 280, 337.
- Damasio, A. R., Castro-Caldas, A., and Levy, A. (1973). In *Progress in Parkinsonism*, ed. D. B. Calne. New York, Raven Press.
- Dunnet, D. L., Brodie, H. K. H., and Goodwin, F. K. (1971). *Clinical Pharmacology and Experimental Therapeutics*, 12, 121.
- Hornykiewicz, O. (1973). In *Treatment of Parkinson's Disease—The Role of Dopa Decarboxylase Inhibitors*, ed. M. D. Yahr. New York, Raven Press.
- McDowell, F. (1972). *Neurology*, 22, 24.
- Markham, C. H. (1972). *Neurology*, 22, 17.
- Reid, J. L., Calne, D. B., Vakil, S. D., Allen, J. G., and Davies, C. A. (1972). *Journal of the Neurological Sciences*, 17, 45.
- Yahr, M. D. (1972). In *Proceedings of the 4th International Symposium on Parkinson's Disease*, ed. J. Siegfried. Bern, Huber.

PRELIMINARY COMMUNICATIONS

Immunoglobulin Deposition in Liver of Patients with Active Chronic Hepatitis and Antibody against Smooth Muscle

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Summary

Liver biopsies from 33 patients with miscellaneous liver diseases were studied by direct immunofluorescence.

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Three patients with active chronic hepatitis possessed a distinctive pattern of staining with anti-IgG conjugate. They were relatively young women and all three possessed the anti-smooth-muscle antibody. In addition, all three were untreated at the time of study. It is suggested that direct immunofluorescence on liver biopsies may help in the investigation of liver disease and that humoral immunity may participate in the pathogenesis of active chronic hepatitis.

Introduction

Active chronic hepatitis was first delineated as a distinct entity because of its association with the lupus erythematosus (L.E.) cell (Joske and King, 1955). Since then several immunological abnormalities have been described but the pathogenesis of this syndrome remains uncertain. The finding of anti-smooth-muscle antibody suggests that humoral immunity could be involved, but the relation between a smooth muscle antigen and

the presence of progressive hepatitis has not been adequately explained (Holborow, 1972; Doniach, 1972). Farrow *et al.* (1971) suggested that the antibody might react with a component of normal liver, but no convincing evidence of in-vivo binding of immunoglobulin has been presented. Immunofluorescent studies on the liver have failed to show any specific change in active chronic hepatitis (Hadziyannis *et al.*, 1969), and have not provided any evidence to support a significant role for antibody in producing the hepatic changes. Nevertheless, the available evidence could be misleading because some authors have failed to distinguish between untreated cases and those receiving steroids or immunosuppressive therapy. It is known that in glomerulonephritis treatment may substantially affect the amount of immunoglobulin which is found in the glomerulus. The present report describes a distinctive pattern of immunoglobulin binding in the liver of three patients with active chronic hepatitis and a high titre of anti-smooth-muscle antibody. None was receiving immunosuppressive therapy at the time of biopsy.

Methods

During a period of about a year, 33 of the liver biopsies performed by one of us (R.A.J.) were studied by direct immunofluorescence and routine histopathology. The tissue obtained was bisected and one half of the biopsy specimen was fixed in 10% buffered formal saline and processed and stained in the routine fashion. The other half was snap-frozen in freezing isopentane after immersion in OCT (multigrade, Lab. Tech.) and was then sectioned at 5 μ in a cryotome. After drying, sections were stained with one of the following fluorescein isothiocyanate (FITC) conjugates: polyvalent antihuman immunoglobulin-FITC (Burroughs Wellcome), and monovalent anti-IgG-FITC, anti-IgM-FITC, anti-IgA-FITC, anti- β 2c-FITC, and anti-fibrinogen-FITC (Hoechst). Representative frozen sections were stained with haematoxylin and eosin to allow correlation between immunofluorescence and the histological findings. The immunological specificity of the staining was tested by determining the "blocking effect" of pretreatment with unconjugated anti-IgG.

The suitability of biopsy specimens for direct immunofluorescence was determined by performing an indirect test with antinuclear factor and polyvalent antihuman immunoglobulin. Antinuclear factor staining was consistently found and was accepted as an indication of antigenic preservation of the tissue. In most cases an additional fragment of the liver biopsy specimen was placed in tissue culture medium, disaggregated by dicing and pipetting, and then stained while viable with polyvalent antihuman immunoglobulin-FITC. Intact cells were identified by means of phase contrast microscopy and then examined by epifluorescence. In this way non-specific staining due to binding to injured cells could be distinguished from true surface staining. This was confirmed by the fact that similar preparatory procedures applied to chicken embryo livers allowed the detection of true surface staining with appropriate antichick antisera (Dawkins and Holborow, 1972).

Sera from all patients were tested by means of indirect immunofluorescence utilizing a composite block of rat liver, rat kidney, rat stomach, rat heart, and mouse stomach. For routine screening the polyvalent antihuman immunoglobulin conjugate was used, but when antibodies were found these were classed by means of monospecific conjugates.

Patients Studied and Results

The 33 patients included 27 with a variety of conditions ranging from alcoholic hepatitis to porphyria. Only one of these 27 showed anti-smooth-muscle antibody. He was a 50-year-old man with chronic Australia antigenaemia and malarial hepatitis. The biopsies from these 27 cases showed no specific pattern of

staining. In two patients with porphyria the characteristic red autofluorescence of the liver was seen but there was no additional abnormality after direct staining. In the remaining 25 cases there was sometimes some poorly defined staining within and around intrahepatic vessels and ducts. This pattern was not found to be of diagnostic significance.

Six patients had the clinical and histological features of active chronic hepatitis. These included liver disease lasting six months or more, jaundice at some stage of the disease, and increased serum levels of alkaline phosphatase, transaminase, and IgG. All six patients were Australia antigen negative. At some stage of the disease liver biopsies from all showed chronic aggressive hepatitis with or without cirrhosis. None had received steroid or immunosuppressive therapy for six months before biopsy.

Three of these patients did not show anti-smooth-muscle antibody, though one possessed anti-cardiac-muscle antibody. Liver tissue from these three showed no consistent pattern of staining on direct immunofluorescence. The remaining three patients were women aged 16, 18, and 21 years at the onset of their disease. All had IgG antibody to smooth muscle in high titre and one also had antinuclear factor. Biopsies from these three cases showed a distinctive pattern of staining. With polyvalent antihuman immunoglobulin-FITC and with monospecific anti-IgG-FITC there was relatively coarse, linear fluorescence along many hepatic cords and patchy polygonal fluorescence around some individual hepatocytes (figs. 1 and 2).

FIG. 1—IgG within the liver of a patient with active chronic hepatitis and anti-smooth-muscle antibody. (Anti-IgG conjugate. $\times 450$.)

The precise localization of the immunoglobulin was not readily apparent because of the limitations of immunofluorescence, the paucity of material, and the architectural distortion associated with active chronic hepatitis and cirrhosis. The staining, however, appeared to be intercellular rather than actually on the hepatocyte surface. The distinctive linear pattern was "blocked" by pretreatment with unconjugated anti-IgG indicating that the deposited material contained IgG. There was no evidence of IgM, IgA, fibrinogen, or complement.

Pretreatment of normal liver biopsy specimens with sera containing the anti-smooth-muscle antibody led to a fine

FIG. 2—As fig. 1, showing linear and pericellular deposition of IgG. ($\times 450$.)

polygonal network as described by others (Holborow, 1972) but did not completely reproduce the coarser patchy staining found in the present three livers by direct immunofluorescence.

Viable cell staining on the liver of the third case with active chronic hepatitis and high titre anti-smooth-muscle antibody failed to show surface-bound IgG.

Discussion

The present evidence indicates that IgG may be deposited in the liver of some patients with active chronic hepatitis. All three positive cases possessed features in common, being young women who were untreated at the time of biopsy. All three possessed high titres of IgG anti-smooth-muscle antibody and

this may indicate that only the antibody-positive patient has hepatic binding of IgG. Since the serum antibody was of the IgG class in all three cases, possibly anti-smooth-muscle antibody itself is bound within the liver. In view of the findings of Holborow (1972), the present observations might be interpreted in terms of an antigen which crossreacts with smooth muscle. If this is the case, however, the failure of anti-smooth-muscle antibody completely to reproduce the characteristic pattern in normal livers suggests that the antigen must be altered in some way in association with active chronic hepatitis. One of several alternative explanations would be that the IgG is an antibody directed against, and reacted with, some liver-specific antigen unrelated to smooth muscle. Another possibility is that the IgG reflects the deposition of circulating antigen-antibody complexes. Further speculation is premature until it has been shown that the staining pattern is due to the deposition of a specific antibody and until the relevant antigen has been identified.

Work is in progress to determine the precise significance and diagnostic specificity of the finding of IgG, but it is suggested that direct immunofluorescence should be performed on liver biopsies. Humoral mechanisms may prove to be important in the pathogenesis of at least antibody-positive active chronic hepatitis. Recent evidence favouring the involvement of cell-mediated immunity should not be considered in isolation (Miller *et al.*, 1972).

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References

- Dawkins, R. L., and Holborow, E. J. (1972). *Journal of Immunological Methods*, 2, 1.
- Doniach, D. (1972). *British Medical Bulletin*, 28, 145.
- Farrow, L. J., Holborow, E. J., and Brighton, W. D. (1971). *Nature*, 232, 186.
- Hadziyannis, S., Feizi, T., Scheuer, P. J., and Sherlock, S. (1969). *Clinical Experimental Immunology*, 5, 499.
- Holborow, E. J. (1972). *Proceedings of the Royal Society of Medicine*, 65, 481.
- Joske, R. A., and King, W. E. (1955). *Lancet*, 2, 477.
- Miller, J., *et al.* (1972). *Lancet*, 2, 296.

Changes in Monosaccharide Content of Mucous Glycoproteins in Ulcerative Colitis

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Summary

Colonic mucus appears to consist of two glycoprotein fractions, one of which contains mannose, whereas the other is mannose-free. The mannose-containing fraction is significantly increased in ulcerative colitis.

Introduction

Mucous glycoproteins are secreted throughout the gastrointestinal tract and have protective and lubricatory functions.

Chemically they consist of a polypeptide chain to which are attached a large number of oligosaccharide units which surround and protect the protein core from proteolysis. The carbohydrate, therefore, is the most important component in determining the physical, chemical, and biological properties of the molecule. This carbohydrate consists of varying proportions of fucose, mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, and sialic acid. Endoscopic mucosal biopsy specimens provide a convenient uncontaminated source of mucus, and their carbohydrate composition is almost exclusively contributed by mucus adherent to the epithelial surface or within goblet cells (Brown *et al.*, 1972). Gas liquid chromatography is capable of analysing the monosaccharide content of the mucous glycoproteins from these small mucosal specimens. Studies were also carried out on first-specimen meconium, which is an abundant source of uncontaminated intestinal secretions containing a large proportion of mucous glycoprotein (D. Fraser and J. R. Clamp, unpublished findings), and this was used for the isolation of reasonable quantities of the two major glycoprotein fractions.

Materials and Methods

Mucosal biopsy specimens were collected at colonoscopy or sigmoidoscopy from normal subjects and from patients with ulcerative colitis, chronic diarrhoea, and infective diarrhoea. All specimens were taken from a constant anatomical site—namely, the rectosigmoid junction—using either the Olympus

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