Lymphocyte cytotoxicity in chronic active hepatitis: effect of therapy and correlations with clinical and histological changes

A. M. G. COCHRANE, A. MOUSSOUROS, A. SMITH, B. PORTMANN,
A. L. W. F. EDDLESTON, AND ROGER WILLIAMS

From the Liver Unit, King's College Hospital and Medical School, Denmark Hill, London

SUMMARY A study of lymphocyte cytotoxicity for rabbit hepatocyte cultures in 15 patients with untreated chronic active hepatitis showed positive results in all cases, both HBsAg positive and negative. After immunosuppressive therapy cytotoxicity became negative and remained negative, in four of nine patients followed serially. In 51 patients established on therapy for periods from three months to 12 years, cytotoxicity was negative in 19 and all patients are currently alive. However, in the remaining 32 patients in whom cytotoxicity was positive there has been a 34% mortality. Cytotoxicity remained persistently positive in 12 of 15 patients followed serially, and persistently negative in seven of nine. Cytotoxicity showed a significant association with histological disease activity, especially the extent of piecemeal necrosis, but not with biochemical tests of liver function, immunoglobulins, or autoantibodies. The basis of this cytotoxicity test is an antibody dependent cell-mediated autoimmune reaction directed against a liver specific protein, and the results suggest that in some cases immunosuppressive therapy is followed by control of this reaction. It may be possible to stop therapy in these patients, but in those in whom the reaction continues, as shown by continuing cytotoxicity, the prognosis is not as good and the use of other drug schedules would seem worthy of trial.

There is now considerable evidence to suggest that cell-mediated autoimmune reactions are involved in the pathogenesis of chronic active hepatitis (Cochrane *et al.*, 1976). Such patients have been shown to be sensitised to a liver specific membrane lipoprotein (LSP) (Miller *et al.*, 1972; Thestrup-Pedersen *et al.*, 1976), and their lymphocytes to be cytotoxic for autologous liver cells (Wands and Isselbacher, 1975; Geubel *et al.*, 1976), Chang cells (Wands *et al.*, 1975; Jacques *et al.*, 1976), and rabbit hepatocytes (Thomson *et al.*, 1974) in tissue culture. In the latter system the reaction can be blocked by LSP, suggesting that it is the major target antigen involved (Thomson *et al.*, 1974) and the ability to demonstrate cytotoxicity after the removal of T cells, and not with T cell enriched fractions, suggests that the reaction is mediated by Fc receptor bearing cells and is therefore another example of antibody dependent cell-mediated cytotoxicity (Cochrane *et al.*, 1976). Antibodies reacting with the antigen on the surface

of rabbit hepatocytes have now been demonstrated in the serum of some patients with antigen negative chronic hepatitis (Tage-Jensen *et al.*, 1977), and repeated immunisation of rabbits with LSP has produced histological changes very similar to chronic aggressive hepatitis (Meyer zum Büschenfelde *et al.*, 1972).

The improvement in survival time following immunosuppressive therapy has been well demonstrated (Cook *et al.*, 1971; Soloway *et al.*, 1972; Murray-Lyon *et al.*, 1973) and in the present study we report the effects of such treatment on the cytotoxicity of peripheral blood lymphocytes for isolated hepatocytes in relation to biochemical histological features.

Methods

PATIENTS

Sixty-six patients with chronic active hepatitis, diagnosed at the time of presentation according to the criteria of de Groote *et al.* (1968), were investigated. Levels of serum aspartate transaminase,

bilirubin, alkaline phosphatase, albumin, total globulin, immunoglobulins, and autoantibodies were determined at the time of the cytotoxicity assay. HBsAg was detectable in the serum of 16 patients by radioimmunoassay (Professor A. Zuckerman, Ausria II, Abbott Laboratories). A liver biopsy had been performed within two weeks of cytotoxicity testing in all 15 patients who were untreated at the time of the initial test, and in 42 of the 51 who were already established on therapy (prednisone 7.5-20 mg daily with or without azathioprine 75 mg or penicillamine 900 mg daily). Each biopsy was assessed by a pathologist as active or inactive without knowledge of the result of cytotoxicity or liver function tests. In addition, the major abnormalities seen in this condition, including those used in the assessment of disease activity, piecemeal necrosis, spotty parenchymal and bridging necrosis, rosette formation, portal infiltration with plasma cells and lymphocytes, Kupffer cell hyperplasia, and bile reduplication were scored from 0-3 (0 absent, 1 minimal, 2 moderate, 3 abundant) and the scores summed to provide an index of total activity.

Techniques

MICROCYTOTOXICITY ASSAY

This assay was carried out using hepatocytes prepared by enzyme digestion of freshly killed rabbit livers and maintained in tissue culture flasks for up to three weeks as described in detail elsewhere (Cochrane *et al.*, 1977). The morphological appearance of the cells, both on light and electron microscopy, their ability to synthesise albumin and to incorporate ^{14}C leucine into acid precipitable material suggested that they were hepatocytes (Hughes *et al.*, 1976). Further evidence of their viability has been shown using metabolic inhibitors and a ^{51}Cr release assay (Hughes *et al.*, 1976). Lymphocytes were prepared from peripheral venous blood by dextran sedimentation, filtration through a cotton wool column (Rocklin *et al.*, 1970), followed by centrifugation over Ficoll triosil (Böyum, 1968). After adjustment to the required concentration, lymphocytes were added to hepatocytes in microtest plates and incubated for 48 hours at 37°C in a 5% CO_2 atmosphere. Detached hepatocytes and lymphocytes were then gently washed clear and the percent cytotoxicity calculated from the formula

$$\frac{\text{average number cells in control well} - \text{average number in test well}}{\text{average number in control well}}$$

$\times 100$. The upper limit of the normal range for cytotoxicity (32%) has been taken as two standard

deviations above the mean value (2.9%) obtained in 35 normal controls (age 17-36 years).

Reproducibility of results

In 120 tests cytotoxicity was estimated from two separate microtest plates using the same preparation of lymphocytes. In 56 (47%) cases the difference in the cytotoxicity values was less than 10% and in 96 (80%) less than 20%. With respect to positive or negative results, 56 of the 120 tests were positive on both occasions and in 50 both fell within the normal range, giving an overall reproducibility rate of 88%. In one normal subject four separate lymphocyte preparations were processed simultaneously from a single blood sample and used in four microtest plates. The mean cytotoxicity of the 16 tests was $7.7 \pm 9\%$ and all fell below 32%, the upper limit of the normal range.

Results

All 15 patients who were untreated at the time of first testing showed significant cytotoxicity. Although the values of cytotoxicity covered a considerable range (from 44-91%) there was no correlation with liver function tests, serum immunoglobulin levels, presence or absence of serum autoantibodies, total biopsy score, or HBsAg status (Table 1).

Serial cytotoxicity tests were performed in nine of these patients following immunosuppressive therapy. Cytotoxicity became negative in four cases within three to six months (Fig. 1) and has remained negative for up to 22 months. In each case the value of aspartate aminotransferase (AST) has remained below twice normal and repeat liver biopsies were considered to be inactive. In the remaining five cases cytotoxicity remained positive. Three of the patients in this group, with persistently abnormal liver function tests, died in liver failure, two of them two months and one two years after treatment was begun. The two remaining cases are still alive at 14 months and 24 months, both having AST values twice normal, although they continue to show histological activity.

Of the 51 patients receiving immunosuppressive therapy at the time of initial testing, 32 (63%) gave positive and 19 (37%) negative cytotoxicity tests. Eleven (34%) of the 32 patients showing cytotoxicity have so far died, whereas there have been no deaths in those with negative results ($\chi^2 = 5.0$, $P < 0.025$). The five year survival (Fig. 2) in patients showing cytotoxicity on the initial test was 55% compared to 100% for those in whom cytotoxicity was negative.

Comparison of patients with positive and negative cytotoxicity at the time of the first test did not

Table 1 Clinical details of patients with untreated chronic active hepatitis

Case	Sex	Age (yr)	Duration of illness (yr)	Drug	AST (IU/l)	Bilirubin (μ mol/l)	Alkaline phosphatase (IU/l)	Albumin (g/l)	Globulin (g/l)	IgG (g/l)	ANA titre	SMA titre	HBsAg +/—	Total biopsy score	% cyto-toxicity	Deaths
1	F	36	60	—	2250	154	207	34	57	2900	10	10	—	21	60	
2	F	19	6	—	1100	170	96	43	42	2000	640	160	—	17	89	
3	F	49	3	—	161	10	125	34	60	1080	—	20	—	13	57	
4	F	51	9	—	1300	240	167	31	51	2270	20	40	—	15	50	
5	F	55	48	—	300	170	151	28	44	1800	—	—	—	13	60	
6	F	62	6	—	1600	78	282	19	62	4200	160	320	—	18	88	+
7	M	30	3	—	177	85	82	39	39	2940	640	160	—	20	45	
8	M	38	6	—	260	53	191	26	40	1500	—	—	—	8	91	
9	M	53	3	—	187	113	139	39	31	1420	—	—	—	14	91	
10	M	67	24	—	280	80	290	20	60	1230	10	10	+	15	44	+
11	M	55	12	—	136	240	135	17	45	1100	—	10	+	16	84	+
12	M	59	48	—	350	50	201	28	59	1770	—	10	+	16	84	+
13	M	52	20	—	115	21	122	45	30	1980	—	—	+	14	46	+
14	M	41	12	—	108	26	102	35	35	1690	—	—	+	11	59	
15	M	41	6	—	130	9	68	44	28	940	—	—	+	14	69

Fig. 1 Lymphocyte cytotoxicity for isolated rabbit hepatocytes in patients with chronic active hepatitis, before treatment (time 0) and serially after immunosuppressive therapy. —: upper limit of normal range. ○: HBsAg positive. ●: HBsAg negative.

reveal any significant differences with respect to age, duration of therapy, ratio of females to males, or HBsAg status. Nor were there significant differences in the mean values of aspartate aminotransferase, bilirubin, alkaline phosphatase, albumin, globulin, immunoglobulin, or presence or absence of autoantibodies determined at the time of cytotoxicity testing (Table 2).

In the 43 treated patients in whom liver histology was available, positive cytotoxicity was associated with an active biopsy in 21 (84%) of 25 cases compared with only three (18%) of 17 negative cases. Similarly there was a close correlation

Fig. 2 Survival curves in patients with treated chronic active hepatitis who showed either positive or negative cytotoxicity.

Table 2 Mean values of liver function tests and immunoglobulins in treated patients with and without significant cytotoxicity

	Cytotoxicity	
	Positive (32)	Negative (19)
Mean age (yr)	43.1 $\pm$ 14	42.8 $\pm$ 14
Duration of therapy (mth)	48.7 $\pm$ 33	44.2 $\pm$ 33
Ratio male:female	1:1	1:1.1
HBsAg positive	7 (22%)	2 (11%)
Aspartate aminotransferase (IU/l)	93	79
Bilirubin (μ mol/l)	27	19
Alkaline phosphatase (IU/l)	125	130
Albumin (g/l)	35	38
Globulin (g/l)	39	38
IgA (g/l)	3.72	3.47
IgG (g/l)	15.28	14.16
IgM (g/l)	1.97	2.29
Autoantibodies (% positive)	65%	50%

between positive cytotoxicity and a high total biopsy score, 19 of 25 patients with positive cytotoxicity scoring 7 or more compared to only three of 17 with negative cytotoxicity ($\chi^2 = 13.5$, $P < 0.0005$). When cytotoxicity was compared with individual histological parameters (Fig. 3), those with positive results showed significantly more piecemeal necrosis ($P < 0.01$, Rank sum test), rosette formation, and spotty parenchymal necrosis ($P < 0.05$, Rank sum test) than those with negative results. There were no other significant differences with respect to other histological features examined.

Serial cytotoxicity tests were performed in 24 treated patients. Of 15 with positive cytotoxicity at the initial test (Fig. 4), 12 (80%) continued to give positive results following continued therapy, although in two the test became negative on one occasion. In contrast, of the nine patients in whom cytotoxicity fell within the normal range on initial testing (Fig. 5), only two (22%), both with values close to the upper limit of the normal range, became positive, a significant difference ($\chi^2 = 5.5$, $P < 0.025$).

Fig. 3 Comparison of the extent of piecemeal necrosis, spotty necrosis and rosette formation, graded 0-3 (0 absent, 1 minimal, 2 moderate, 3 abundant) in patients with positive (closed column) and negative (open column) cytotoxicity.

Fig. 4 Serial lymphocyte cytotoxicity tests in patients already established on immunosuppressive therapy who showed positive cytotoxicity on first testing (symbols as for Fig. 1).

Fig. 5 Serial lymphocyte cytotoxicity tests in patients already established on immunosuppressive therapy who showed negative cytotoxicity on first testing.

Discussion

Although cytotoxicity was demonstrated in all patients with untreated chronic active hepatitis, there was a wide variation in the values recorded. This is unlikely to be solely due to variations in the experimental technique as shown by the results of the reproducibility studies. The lack of a correlation between the degree of cytotoxicity and liver function tests or the degree of histological activity is not surprising. The explanation may relate to the fact that the ability to demonstrate cytotoxicity *in vitro* presumably depends on the *in vivo* absorption of complexes of liver specific protein and its antibody to Fc-receptor bearing cells (Cochrane *et al.*, 1976). Support for this concept comes from recent studies in which we have shown that normal lymphocytes develop cytotoxicity for hepatocytes after incubation in serum from patients with chronic active hepatitis (Gonzalez *et al.*, 1978). Competition for these receptor sites by other immune complexes present in the serum, perhaps as a consequence of the liver damage, will therefore modify the degree of cytotoxicity.

Although in the patients already established on immunosuppressive therapy there was a close association between the ability to demonstrate cytotoxicity and an active liver biopsy (score of 7 or more), there was again no correlation between the degree of cytotoxicity and biopsy score in those showing positive cytotoxicity. As the cytotoxic reaction is thought to be antibody mediated (Cochrane *et al.*, 1976), the striking association between the finding of cytotoxicity and the pressure of piecemeal necrosis may be due to periportal hepatocytes adsorbing the majority of antibody to liver-specific protein, and thus being the most susceptible to destruction by Fc-receptor bearing cells. This may be why periportal or piecemeal necrosis is such a hallmark of this disease in both HBsAg negative and positive cases.

In contrast with the association between positive cytotoxicity and certain histological features there were no significant differences in liver function tests, immunoglobulins, or frequency of auto-antibodies between those with and without lymphocyte cytotoxicity. We have suggested that immunoglobulin levels may be largely genetically

determined, while autoantibodies may be markers of an inherited autoimmune diathesis (Galbraith *et al.*, 1976) and neither would therefore directly reflect disease activity. As liver function tests reflect the synthetic and metabolic capacity of the liver as a whole and cytotoxicity reflects only one specific immunological reaction, it would be surprising if there were an association between any of these parameters.

The factors determining the variability in the response to treatment are largely unknown, but it may be helpful in planning the management of these patients to construct a working hypothesis. Thus, it seems likely that the conventional immunosuppressive drugs in the doses used by most authors are not truly immunosuppressive but are principally exerting an anti-inflammatory action (Denman *et al.*, 1970). We have proposed that the autoimmune reaction in chronic active hepatitis is maintained by a constant helper T-cell stimulus of autoreactive B cells and a partial defect in suppressor T-cell activity (Eddleston and Williams, 1974). In HBsAg negative chronic active hepatitis the latter defect would predominate, but Allison *et al.* (1971) have pointed to the potential importance of altered self antigens, released at the site of tissue injury, acting to promote a helper T-cell response. Corticosteroids with their action to reduce lysosomal enzyme release would be potent inhibitors of this process, and the reduction in the formation of the altered self antigens could be sufficient to allow a weak suppressor T-cell system to regain control. In HBsAg positive chronic active hepatitis, on the other hand, the self antigens are altered by viral products and anti-inflammatory drugs would not directly reduce formation, and the stimulus for autoimmunity would persist. This may explain the relatively poor response to treatment in the HBsAg positive cases reported by Schalm *et al.* (1976). We found all but one of our antigen positive cases to be histologically active with persistently positive cytotoxicity and half have so far died. In this group, and in the HBsAg negative cases with postulated severe suppressor T-cell defects, more intense therapy with a truly immunosuppressive action might be needed and it is of interest that Schalm was able to establish control in some of his unresponsive cases by doubling the dose of prednisone or azathioprine.

References

- Allison, A. C., Denman, A. M., and Barnes, R. D. (1971). Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet*, **2**, 135-140.
- Böyum, A. (1968). Isolation of leucocytes from human blood. *Scandinavian Journal of Clinical and Laboratory Investigation*, **21**, Suppl 97, 9-29.
- Cochrane, A. M. G., Moussourous, A., Portmann, B., McFarlane, I. G., Thomson, A. D., Eddleston, A. L. W. F., and Williams, R. (1977). Lymphocyte cytotoxicity for isolated hepatocytes in alcoholic liver disease. *Gastroenterology*, **72**, 918-923.
- Cochrane, A. M. G., Moussourous, A., Thomson, A. D., Eddleston, A. L. W. F., and Williams, R. (1976). Antibody-dependent cell-mediated (K cell) cytotoxicity against isolated hepatocytes in chronic active hepatitis. *Lancet*, **1**, 441-444.
- Cook, C. G., Mulligan, R., and Sherlock, S. (1971). Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. *Quarterly Journal of Medicine*, **40**, 159-185.
- Denman, E. J., Denman, A. M., Greenwood, B. N., Gall, D., and Heath, R. B. (1970). Failure of cytotoxic drugs to suppress immune responses of patients with rheumatoid arthritis. *Annals of Rheumatic Diseases*, **29**, 220-231.
- Eddleston, A. L. W. F., and Williams, R. (1974). Inadequate antibody response to HBsAg or suppressor T-cell defect in development of active chronic hepatitis. *Lancet*, **2**, 1543-1545.
- Galbraith, R. M., Eddleston, A. L. W. F., Williams, R., Webster, A. D. B., Pattison, J., Doniach, D., Kennedy, L. A., and Batchelor, J. R. (1976). Enhanced antibody responses in active chronic hepatitis: relation to HLA-B8 and HLA-B12 and porto-systemic shunting. *Lancet*, **1**, 930-934.
- Geubel, A. P., Keller, R. H., Summerskill, W. H. J., Dickson, E. R., Tomasi, T. B., and Shorter, R. G. (1976). Lymphocyte cytotoxicity and inhibition studied with autologous liver cells: observations in chronic active liver disease and the primary biliary cirrhosis syndrome. *Gastroenterology*, **71**, 450-456.
- Gonzalez, C., Cochrane, A. M. G., Jensen, D., McFarlane, I. G., Eddleston, A. L. W. F., and Williams, R. (1978). The role of antibody to liver specific protein (LSP) in lymphocyte cytotoxicity for rabbit hepatocytes in chronic active hepatitis. (In preparation).
- de Groote, J., Desmet, V. J., Gedigk, P., Korb, G., Popper H., Poulsen, H., Scheuer, P. J., Schmid, M., Thaler, H., Uehlinger, E., and Wepler, W. (1968). A classification of chronic hepatitis. *Lancet*, **2**, 626-628.
- Hughes, R. D., Cochrane, A. M. G., Thomson, A. D., Murray-Lyon, I. M., and Williams, R. (1976). The cytotoxicity of plasma from patients with acute hepatic failure to isolated rabbit hepatocytes. *British Journal of Experimental Pathology*, **57**, 348-353.
- Jacques, E. A., deVilliers, D., Crimmins, F., Thomas, H. C., and Sherlock, S. (1976). Effect of prednisolone and azathioprine on K cell cytotoxicity in chronic active hepatitis. *Gastroenterology*, **71**, 913 (abstract).
- Meyer zum Büschenfelde, K. H., Kössling, F. K., and Miescher, P. A. (1972). Experimental chronic active hepatitis in rabbits following immunization with human liver proteins. *Clinical and Experimental Immunology*, **11**, 99-108.
- Müller, J., Smith, M. G. M., Mitchell, C. G., Reed, W. D., Eddleston, A. L. W. F., and Williams, R. (1972). Cell-mediated immunity to a human liver-specific antigen in patients with active chronic hepatitis and primary biliary cirrhosis. *Lancet*, **2**, 296-297.
- Murray-Lyon, I. M., Stern, R. B., and Williams, R. (1973). Controlled trial of prednisone and azathioprine in active chronic hepatitis. *Lancet*, **1**, 735-737.
- Rocklin, R. E., Meyers, O. L., and David, J. R. (1970). An *in vitro* assay for cellular hypersensitivity in man.

- Journal of Immunology*, 104, 95-102.
- Schalm, S. W., Ammon, H. V., and Summerskill, W. H. J. (1976). Failure of customary treatment in chronic active liver disease: causes and management. *Annals of Clinical Research*, 8, 221-227.
- Soloway, R. D., Summerskill, W. H. J., Baggenstoss, A. H., Geall, M. G., Gitnick, G. L., Elveback, L. R., and Schoenfeld, L. J. (1972). Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology*, 63, 820-833.
- Tage-Jensen, U., Arnold, W., Dietrichson, O., Hardt, F., Hopf, U., Meyer zum Büschenfelde, K. H., and Nielsen, J. O. (1977). Liver-cell-membrane autoantibody specific for inflammatory liver diseases. *British Medical Journal*, 1, 206-208.
- Thestrup-Pedersen, K., Ladefoged, K., and Andersen, P. (1976). Lymphocyte transformation test with liver-specific protein and phytohaemagglutinin in patients with liver disease. *Clinical and Experimental Immunology*, 24, 1-8.
- Thomson, A. D., Cochrane, A. M. G., McFarlane, I. G., Eddleston, A. L. W. F., and Williams, R. (1974). Lymphocyte cytotoxicity to isolated hepatocytes in chronic active hepatitis. *Nature*, 252, 721-722.
- Wands, J. R., and Isselbacher, K. J. (1975). Lymphocyte cytotoxicity to autologous liver cells in chronic active hepatitis. *Proceedings of the National Academy of Sciences*, 72, 1301-1303.
- Wands, J. R., Perrotto, J. L., Alpert, E., and Isselbacher, K. J. (1975). Cell-mediated immunity in acute and chronic hepatitis. *Journal of Clinical Investigation*, 55, 921-929.

The March 1978 Issue

THE MARCH 1978 ISSUE CONTAINS THE FOLLOWING PAPERS

Editorial: Vale: Sir Francis Avery Jones

Selection of patients for bile diversion surgery: use of bile acid measurement in fasting gastric aspirates A. M. HOARE, A. MCLEISH, H. THOMPSON, AND J. ALEXANDER-WILLIAMS

Measurement of bile acids in fasting gastric aspirates: an objective test for bile reflux after gastric surgery A. M. HOARE, M. R. B. KEIGHLEY, B. STARKEY, AND J. ALEXANDER-WILLIAMS

Serum prolactin in liver disease and its relationship to gynaecomastia MARSHA Y. MORGAN, A. W. JAKOBOVITS, MARION B. R. GORE, M. R. WILLS, AND SHEILA SHERLOCK

Glucagon therapy in acute pancreatitis H. K. DÜRR, D. MAROSKE, O. ZELDER AND J. CH. BODE

Exocrine pancreatic secretion and immunoreactive secretin (IRS) release after intraduodenal instillation of bile in man M. OSNES, L. E. HANSSEN, O. FLATEN, AND J. MYREN

Vagal stimulation and its role in eliciting gastrin but not glucagon release from the isolated perfused dog stomach P. J. LEFEBVRE, A. S. LUYCKX, AND A. H. BRASSINNE

Lymphocytotoxicity test against rabbit hepatocytes in chronic liver diseases A. FACCHINI, G. F. STEFANINI, M. BERNARDI, F. MIGLIO, G. GASBARRINI, AND G. LABÖ

Effect of continuous jejunal perfusion of elemental and complex nutritional solutions on pancreatic enzyme secretion in human subjects N. VIDON, P. HECKETSWEILER, J. BUTEL, AND J. J. BERNIER

Nitrate studies in oesophageal cancer A. B. LOWENFELS, A. J. TUYNS, E. A. WALKER, AND A. ROUSSEL

Increased sensitivity of gastrin release to adrenaline in duodenal ulcer O. BRANDSBORG, N. J. CHRISTENSEN, N. A. LØVGREEN, M. BRANDSBORG, AND J. F. REHFELD

Serum level of immunoreactive gastrin: influence of kidney function R. HÄLLGREN, F. A. KARLSSON, AND G. LUNDQVIST

Plasma cyclic AMP levels during a secretin-caerulein pancreatic function test in liver and pancreatic disease M. ADLER, P. ROBBERECHT, M. G. POITEVIN, AND J. CHRISTOPHE

Calcium carbonate in human gallstones and total CO₂ in bile D. JUNE SUTOR AND LYNETTE I. WILKIE

Immune-complex mediated colitis in rabbits H. J. F. HODGSON, B. J. POTTER, J. SKINNER, AND D. P. JEWELL

Serum and faecal lysozyme in inflammatory bowel disease H. J. KLASS AND G. NEALE

Digestion of native collagen in the gut MARGARET L. R. HARKNESS, R. D. HARKNESS, AND MARY F. VENN

Short report Use of ERCP in the diagnosis of internal pancreatic fistula M. SOTTOMAYOR, R. NOM CHONG, AND M. DAWSON

Notes and activities

Book reviews

Copies are still available and may be obtained from the PUBLISHING MANAGER, BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, price £2.75, including postage