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Suppressor T cell activity and antibodies to alcohol altered hepatocytes

Professor MacSween and his colleagues have described antibodies to alcohol altered rabbit hepatocytes in the sera of patients with alcoholic liver disease.[1 2] This finding suggests an immunological mechanism in the pathogenesis of alcoholic liver disease. Recently, it has been postulated that a decrease in suppressor T cell activity may contribute to the abnormal immune response found in alcoholic hepatitis and alcoholic cirrhosis.[3]

To investigate the contribution of cellular immunoregulation in the production of antibodies against alcohol altered hepatocytes we studied the suppressor of T cell function and the presence of antibodies to alcohol altered rabbit hepatocytes in 78 alcoholic patients with or without liver disease and in 35 healthy controls. The suppressor cell activity was induced by concanavalin A and measured by the inhibition of mitogen blast transformation. The antibodies to alcohol altered rabbit hepatocytes were investigated by indirect immunofluorescence with hepatocytes isolated from rabbits previously treated with alcohol.

Antibodies to alcohol altered rabbit hepatocytes were shown in the sera of 87% of patients with alcoholic hepatitis, 50% of patients with inactive cirrhosis, 26% of the alcoholics without liver disease, and 4% of the controls. There was also a significant reduction in suppressor T cell activity in patients with alcoholic hepatitis when compared with the control group ($p < 0.001$). No relation was found, however, between the presence of alcohol altered rabbit hepatocyte antibodies and the reduction in suppressor T cell activity.

This study suggests that these phenomena are unrelated and are probably due to independent underlying causes.

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Continuous marker test for fat absorption

Teh Lip Bin *et al*[1] have provided further evidence of the value of the single sample test for stool fat using copper(I)thiocyanate as marker, while recognising that the test needs further development. One simplification would be to give the daily oral dose of marker as 1 mmol (121·6 mg; Koch-Light Laboratories, please note), containing 1 mmol of copper; thus the awkward 1·17 factor in their calculation would become unnecessary.

There is a printing error in the paper, however, and 1·17 should be 1·97.

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Selective Kirchner medium in the culture of specimens for mycobacteria

Mitchison *et al*[1] have recommended a combination of two Löwenstein-Jensen slopes, (one with and one without pyruvate) and a selective Kirchner medium as the most efficient procedure for isolating *Mycobacterium tuberculosis* from specimens other than sputum.

We would like to record our experience of using this system in the examination of 2471 clinical specimens (including sputum) between 1981 and 1983. The media used were Löwenstein-Jensen (LJ); Löwenstein-Jensen with 0·5% sodium pyruvate (LJ+P); liquid Kirchner (NSK); and selective liquid Kirchner (SK). All media were freshly prepared in the laboratory according to the formulation of Marks.[2] The SK medium contained 1 ml/l calf serum and the same antibiotics as those used by Mitchison *et al*, although the concentrations used were different in some cases (amphotericin 10 mg/l, trimethoprim 15 mg/l, polymyxin B 200 units/l, and carbenicillin 50 mg/l). Specimens were inoculated into the SK medium without prior decontamination. Before inoculation into any other media, however, they were treated by a modified Petroffs technique.[3] All cultures were incubated at 37°C for 6 weeks and examined weekly for evidence of growth. At the end of 6 weeks, the NSK and the SK media were subcultured to LJ+P slopes and incubated for a further 4 weeks.

The total number of specimens examined by us (2471) was comparable to that examined by Mitchison *et al* (2949), but the distribution of the various types of specimens was rather different. For example, sputa accounted for 24·6% of our specimens but were not included in the study by Mitchison *et al*, while the figures were 16% and 30% respectively for pleural fluids and 32% and 45% respectively for urine samples. In our series a positive ZN film was shown in 49 specimens (1·9%) as opposed to 22 (0·7%) of the specimens examined by Mitchison *et al*. The figures for positive cultures were 55(2·3%) and 52(1·8%) respectively. We also found the SK medium to be of value in increasing the yield of positive cultures above that obtained with use of LJ and LJ+P media. In our case this represented 11 further positive cultures (an increase of 20%). This is less than the 36% increase obtained by Mitchison *et al*, but the difference may be attributable to the variation in specimen types and the higher incidence of ZN positive specimens in our series.

The table shows the number of positive cultures from the various types of specimens in relation to the culture media used. Of the seven isolates obtained only from SK medium, the distribution by specimen types was urine (2), sputum (2), cerebrospinal fluid (1), pleural fluid (1), and brain tissue (1). No positive results were obtained in the other media in those cases where a negative result was obtained with SK medium.

We believe that the benefit of incorporating SK media into routine procedure is considerable. The medium has been used successfully without prior decontamination of specimens and the procedure does not greatly increase laboratory costs or work

Numbers of cultures obtained on various media

Specimen	No of specimens	ZN positive	No of positive cultures on:			
			LJ	LJ+P	NSK	SK
Urine	783	7 (0·9%)	7	7	7	9
Sputum	608	18 (3·0%)	9	16	17	19
Cerebrospinal fluid	123	1 (0·8%)	2	2	2	3
Gastric washings	10	1 (10%)	1	1	1	1
Pleural fluid	395	0	1	1	1	2
Pus	240	9 (3·8%)	7	7	7	7
Tissues	312	13 (4·2%)	9	10	13	14
Total	2471	49	36	44	48	55

LJ = Löwenstein-Jensen.
LJ+P = Löwenstein-Jensen with 0·5% sodium pyruvate.
NSK = liquid Kirchner.
SK = selective liquid Kirchner.
ZN = Ziehl-Neelsen.

load. The use of a selective Kirchner medium in routine isolation does have the disadvantage that a culture result is not possible for at least nine weeks after initial incubation. In those cases where only the selective Kirchner was positive the number of viable mycobacteria present in the original was very low. The selective Kirchner also has the advantage that it can act as an enrichment medium, allowing the mycobacteria to increase at the expense of any other bacteria present. In those specimens where large numbers of mycobacteria are present and contaminating bacteria absent, quicker results will be obtained with conventional culture media. The use of a selective Kirchner should therefore be seen as an addition rather than a replacement.

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Measurement of creatine kinase by reflectance spectroscopy and reagent strips—effect of EDTA

In our recent assessment of the measurement of creatine kinase activity,[1] we showed considerable inhibition by EDTA of both the Seralyzer and the LKB/optimised reagent assays.

Although the property of EDTA to sequester bivalent metal cations required for the activation of creatine kinase is well recognised, the concentration of EDTA present in most commercial blood collection tubes (about 1·5 mg/ml blood) was not thought to be high enough to cause interference with these creatine kinase assays.

In the original study, Sterilin EDTA blood collection tubes were used. We have repeated this study using commercial EDTA tubes from three sources: a new batch from Sterilin and tubes from Trident and Labco, all containing essentially the same concentration of EDTA. Creatine kinase assays were performed on the Seralyzer and with the BCL optimised (37°C) reagents on both the LKB reaction rate analyser and the Centrifichem analyser. No appreciable inhibition by EDTA was found with the Seralyzer or with either of the two liquid chemistries with a wide range of creatine kinase activities.

The EDTA tubes used in the original study are now thought to have been rather old, although we have been unable to determine the date of manufacture.

Three conclusions may be drawn from these studies:

1 The Seralyzer creatine kinase system and the conventional liquid chemistries studied do not suffer interference from most commercial EDTA blood collection tubes.

2 EDTA tubes of recent purchase only should be used.

3 In order to minimise any increase in EDTA concentration, these collection tubes should be filled with the recommended volume of blood.

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Is enrichment culture necessary for the isolation of *Campylobacter jejuni* from faeces?

We were interested to read the discussion of the relevance of enrichment culture to the isolation of campylobacters from human faeces.[1] We have used enrichment in modified Preston broth[2] as part of our method for campylobacter isolation since August 1982. Since that time 33 campylobacter isolations have been made from about 2000 specimens of human faeces in this laboratory. Of these 33, direct plating on to Preston[3] and Skirrows'[4] agars failed to show the presence of campylobacters in six specimens, and isolation was achieved only by the use of enrichment.

Of the six samples which were positive by enrichment culture only, two were from patients whose symptoms had declined and therefore represent "convalescent specimens" as described by Hutchinson and Bolton.[1] The remaining four specimens, however, were from a single patient and were taken before the onset of diarrhoea (one) and during the acute phase of the illness (three), which lasted 12 days.

We therefore support the view of Hutchinson and Bolton[1] that enrichment culture has little effect on the isolation of campylobacters from most patients with acute diarrhoea, provided that a good selective agar is used and that the delay in culturing specimens is minimal. It should be recognised, however, that in some cases the use of enrichment culture is necessary for the isolation of campylobacters from patients with acute campylobacter enteritis.

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