avoidance of protein precipitation and bilirubin interference without resort to complex equipment is a considerable advantage. The relation between pH and nitrite is in agreement with results obtained using different methodology.[1-3]

In conclusion, the suggested role of nitrite in human gastric cancer has led to a series of studies on gastric juice nitrite. We believe that the method described provides a simple and rapid analytical technique suitable for performing such studies.

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# Letters to the Editor

## Effect of an evacuated blood collection system on coagulation screening tests

An evacuated blood collection system for routine blood sampling is now being introduced into many British hospitals as a labour and cost saving technique.[1] The use of these evacuated tubes for coagulation studies, however, has been severely criticised and even condemned.[2,3] In most cases it is not convenient or desirable for a hospital to have to maintain a second routine blood taking method just for coagulation investigations. We have therefore compared samples drawn with venous cannula and syringe with those obtained by an evacuated tube technique.

Blood specimens were collected from 30 apparently healthy adult volunteers and from 19 patients regularly attending an anticoagulant clinic, who were selected at random. Venous blood was obtained by clean venepuncture with a 21 G butterfly needle (Argyle; St Louis, USA) and a disposable polystyrene syringe. Nine volumes of blood were added to one volume of 0·105 M trisodium citrate in a polystyrene plastic tube (Brunswick; Sherwood Medical, Co Antrim, N Ireland). Blood was also drawn through the same 21 G butterfly by means of a multiple sample luer adapter into a sterile siliconised evacuated tube (Vacutainer; Becton Dickinson, London) containing 0·105 M buffered sodium citrate. The present study was performed with one batch of Vacutainer tubes, no 676624, lot no I E 114, before their expiry date. The blood was immediately centrifuged at 2000 *g* for 15 min and the platelet poor plasma was tested within 60 min of venesection. Both types of tubes remained stoppered until tested. The thrombin and prothrombin times (using the Manchester comparative reagent)[4] and the activated partial thromboplastin time (APTT) with kaolin[5] were measured in duplicate on each sample by standard manual techniques. The differences between the results of the three tests performed on plasma obtained by the two sampling techniques in the normal and anticoagulant groups are shown in the Table.

*Differences between results of the coagulation tests on plasma obtained by the two techniques—plastic syringe and tube and an evacuated tube Vacutainer system*

| *Coagulation test* | *Normal group (n = 30)* | | | *Anticoagulation group (n = 19)* | | |
|---|---|---|---|---|---|---|
| | *Mean difference* | *SD* | *p* | *Mean difference* | *SD* | *p* |
| Thrombin time (s) | −0·55 | 0·94 | 3·31 | −0·50 | 1·01 | 0·05 |
| Prothrombin time (s) | 0·13 | 0·62 | 0·24 | −0·11 | 1·06 | 0·67 |
| Activated partial thromboplastin time (s) | 1·63 | 1·66 | <0·001 | 3·16 | 3·23 | <0·001 |

The evacuated tubes gave an acceptable correlation with the plastic tubes for the prothrombin time and thrombin time in both the normal and anticoagulant groups; but there was a significantly longer APTT with the evacuated samples in both groups ($p < 0·001$). The lengthening of the APTT ranged up to 4 s in the normal group and 7 s in the anticoagulant group. This was sufficient to make six of the 30 normal volunteers' APTTs abnormal as defined by our laboratory range. Previous reports using evacuated Vacutainer tubes have shown a shortening of the APTT related to an old stopper formulation containing isoprene[2] and prolongation of the APTT after storage of samples in unstoppered tubes before testing.[6] We used evacuated tubes with neobutyl rubber stopper formulation[7] and performed all the tests from stoppered tubes. The precise cause of the variable prolongation of the APTT which we observed remains uncertain, but it may result from adsorption or inactivation of intrinsic contact coagulation factors.

With several commercial evacuated tubes for blood collection now being widely introduced into hospitals in the UK it is important that their effects on the coagulation system are compared with the standard syringe and polystyrene plastic tube method before they are considered for routine use.


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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 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