Letters to the Editor

Phenazine methosulphate and the nitroblue tetrazolium macroreaction for recent myocardial infarction

The use of 5-methylphenazinium methylsulphate (phenazine methosulphate) as an intermediate electron carrier for coupling the production of NADH to the reduction of tetrazolium salts to coloured formazans is well established. Derias and Adams recently investigated its possible benefit in the nitroblue tetrazolium reaction for gross identification of recent myocardial infarction and concluded that it results in false staining of areas of myocardial damage. Rosalki has pointed out the wide range of phenazine methosulphate concentrations which have been used in enzyme histochemistry—0-0007 mg/ml to 1.3 mg/ml. I would like to report the beneficial use of phenazine methosulphate in the nitroblue tetrazolium macroreaction for detecting myocardial infarction when used in low concentration and short incubation.

Fifteen hearts were examined: 10 from patients with definite evidence of recent myocardial infarction leading to death, two from patients with non-ischaemic cardiomyopathy, one from a patient with valvular heart disease, and two from young women dying from a cerebral tumour and haemorrhagic pancreatitis. They were removed at necropsy between two and 48 hours after death and then rinsed, frozen, and cut transversely into roughly 1 cm thick slices. Heart slices were then incubated for 10–20 min in a solution of nitroblue tetrazolium (50 mg/100 ml) in Sorenson’s phosphate buffer (pH 7.4, 0.1 M) at 37°C, according to the method of Nachlas and Shnitka. Sufficient incubating medium was used to ensure the heart slices were completely covered, thereby preventing atmospheric oxygen from competing for liberated electrons. Consecutive slices in four hearts were stained to compare the endogenous substrate nitroblue tetrazolium method with the effects of adding specific substrate creatine phosphate 100 mg/100 ml, coenzyme NAD 50 mg/100 ml, or phenazine methosulphate 2.5 mg/100 ml to the basic nitroblue tetrazolium incubating medium. Slices of a further four hearts were used to investigate the effect of adding concentrations of phenazine methosulphate varying between 1 mg and 10 mg/100 ml to the nitroblue tetrazolium medium, and incubating between 10 and 20 min. As phenazine methosulphate is photolabile, incubation was carried out in darkness. After incubation the slices were rinsed and fixed in 10% formalin.

The basic nitroblue tetrazolium method stained normal, myopathic, and non-infarcted myocardium blue-purple after 20 min. The border zone of the unstained infarcted areas was not as clearly defined, nor was the staining as uniformly darkly contrasting as when phenazine methosulphate was added in a concentration of 2.5 mg/100 ml to slices removed within a few hours of death and incubated for just 10 min (Fig. 1). The addition of specific substrate creatine phosphate showed no improvement on the basic method. NAD accelerated staining and delineated the infarcts but resulted in false staining of fat and infarct within 10 min and discoloured the incubating medium (Fig. 1). Higher concentrations of phenazine methosulphate (5–10 mg/100 ml) led to dark discolouration of the incubating medium and reduced staining of infarcted areas if incubation was continued for 20 min (Fig. 2).

The best delineation of infarcts was achieved by adding phenazine methosulphate to the nitroblue tetrazolium incubating medium in a low concentration (2.5 mg/100 ml) and staining for 10 min. The benefit of adding phenazine methosulphate in this way was seen whether necropsy was performed two or 30 hours after death; no necropsy in this study was performed more than 48 hours after death. This technique had now been used successfully to stain slices of a further 19 hearts from patients dying from recent myocardial infarction and examined within four hours of death. The dark contrast of the normal myocardium as compared with the infarcted myocardium and the clarity of the infarct border aid infarct sizing by stereological analysis for comparison with 201-thallium tomographic data acquired during life.

Phenazine methosulphate was accepted in histochemical practice in 1956. Pearse has described its ability to accelerate the succinic dehydrogenase and diaphorase reactions using nitroblue tetrazolium, but both accentuation and depression of stain-
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References


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

We were interested to read that Hutchinson and Bolton1 considered that direct culture on a good selective agar was adequate for the isolation of Campylobacter jejuni and C. coli from patients with acute diarrhoea. We came to the same conclusion after carrying out a similar study, which was prompted by the report of Ribeiro et al,2 who increased their isolation rate by a surprising 74% with Preston broth enrichment.

For direct culture we used Columbia agar (Oxoid CM 331) containing Skirrow3 antibiotic supplement (Oxoid SR 69). Plates were incubated at 42-43°C for up to 48 h in anaerobic jars (without catalysts) which had each been primed with a gas-generating envelope (Oxoid BR 38) and then immediately evacuated to 550 mmHg below atmospheric pressure. In addition, a portion of each sample was cultured in Preston enrichment broth4 for 24 h before subculturing to the selective agar as described above.

These methods were used to examine 2231 faecal samples from patients with gastrointestinal symptoms encountered over one year. C jejuni or C. coli was isolated from 190 samples (8.5%). Of these, 186 were positive by both direct plate culture and enrichment and 10 were positive by enrichment only—an increase of 5.6% over the number positive by direct culture.

All but two of the 10 samples positive only by enrichment were either of formed consistency (five samples) or stale (three samples were three days old).

Provided one's direct culture methods are sound, there is little to be gained from the routine use of enrichment cultures for the isolation of campylobacters from clinical specimens. Our policy now is to set up enrichment cultures only on diarrhoea faeces that are more than 24 h old and on formed or semi-formed faeces from patients with a stated history of abdominal pain or fever. Formed faeces from patients alleged to have diarrhoea on request forms (about one third of our intake and double the number of many other laboratories) are probably not worth culturing at all, let alone by enrichment methods.

We thank the laboratory staff for carrying out the extra work that this study entailed.

MB SKIRROW

J BENJAMIN

Department of Microbiology
Worcester Royal Infirmary
Worcester WR1 3AJ

References