fluorescence of ordinary glass slides is not readily visible. With incident light illumination the fluorescent area of the slide lies immediately below the specimen and may obscure specific fluorescence. The primary filter GG 420/4 mm reduced this interference. Non-fluorescent multi-specimen slides are made by Hendley & Co, Buckhurst Hill, Essex.

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Improved mountant for immunofluorescence preparations

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Fluorescence emission of fluorescein isothiocyanate (FITC) from labelled antibody in microscopical preparations may be influenced by the characteristics of the mounting medium, in particular, its pH, its ionic strength, its viscosity, and the presence of quenching agents (Cherry, 1970). Fluorescence emission is greater at alkaline than at acid pH (Hiramoto, Bernecky, Jurand, and Hamlin, 1964). Pital and Janowitz (1963) claimed maximum fluorescence with glycerol buffered at pH 9·0 but Nairn (1969) recommended a slightly lower pH (8·6) so as to avoid impairment of immunological reactivity and fine tissue morphology. In a quantitative study of emission from FITC bound to cells mounted in buffered glycerol, Jongsma, Hijmans, and Ploem (1971) observed maximum fluorescence at pH 8·5. Small variations around this pH did not significantly influence fluorescence emission.

In spite of this well documented information it is still common practice to use a mounting medium which consists of glycerol and phosphate-buffered saline at pH 7·1. Furthermore, such a mountant does not give firm adhesion between the cover glass and the microscope slide.

A semi-permanent medium containing polyvinyl alcohol grade 51-05 Elvanol (PVA)[1] and glycerol was described by Rodriguez and Deinhardt (1960). This alternative to buffered glycerol solidifies on drying without trapping air bubbles provides an adhesive film between the cover glass and the microscope slide, and was used by Taylor, Heimer, Lea, and Tomlinson (1964) to mount immunofluorescence preparations of *Shigella sonnei*. Several semi-permanent mountants containing PVA were later evaluated in immunofluorescence studies by Thomason and Cowart (1967). The method of preparing the mountant described by Rodriguez and Deinhardt includes mechanical stirring for 32 hours so as to dissolve the PVA and centrifugation at 12 000 rpm to clear the solution. The final pH of this

[1]Du Pont Co, (UK) Ltd,
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mountant is between 6 and 7. On storage, some batches of mountant prepared in this manner, as well as some commercially obtainable mountants containing PVA, become cloudy with sedimentation, thicken and even solidify. The purpose of this technical note is to describe a simple method for making an improved reagent of this type. This mountant (containing tris buffer pH 8·5 and designated A here) has a good shelf life, remains clear, and does not thicken after 12 months' storage in air-tight bottles or even during routine use.

We have compared fluorescence emission from single bacilli in preparations mounted in mountant (A) with that obtained from similar preparations mounted in glycerol and buffer at pH 8·5 (designated B here) and at pH 7·1 (designated C here).

Materials and Methods

BUFFER SOLUTIONS

Tris (0·0995 M) pH 8·5

Dissolve 2·42 g (hydroxymethyl) methylamine (Puriss) powder (tris)[2] in 95·0 ml demineralized water. Add 3·0 ml N/1 HCl and continue adding dropwise until the solution is pH 8·5. The final volume is made up to 100 ml with demineralized water.

Phosphate-buffered saline (NaCl 0·145 M phosphate 0·01 M) pH 7·1

NaCl 8·5 g
Na_2HPO_4 (anhydrous) 1·07 g
$NaH_2PO_4 2H_2O$ 0·39 g
Distilled water to 1000 ml

PREPARATION OF THE MOUNTANT (A)

Weigh 3 g analytical quality glycerol in a Sterilin[3] universal container with a conical bottom, add 1·2 g PVA, and stir well without spreading the PVA powder onto the side of the container. Add 3 ml demineralized water, stir, and leave for four hours at room temperature.

To prevent the formation of lumps it is important to mix the glycerol and PVA completely before adding the water.

Add 6 ml tris buffer solution to the mixture and place the universal container in a water bath at 50°C.

Leave for 10 minutes with occasional agitation so as to dissolve the PVA.

Finally, clear the reagent by centrifugation at 3000 rpm for 15 minutes.

A preservative such as merthiolate is not added.

BUFFERED GLYCEROL MOUNTANTS (B) AND (C)

B 90 g glycerol 10 ml tris buffer pH 8·5
C 90 g glycerol 10 ml phosphate-buffered saline pH 7·1

MICROSCOPICAL PREPARATIONS

Smears of an 18-hour culture of *Eschericia coli* 026:B6 were made on microscope slides then dried

[2] Koch-light Laboratories Ltd, Colnbrook, Bucks, England.

[3] Sterilin Ltd, Richmond, Surrey, England.

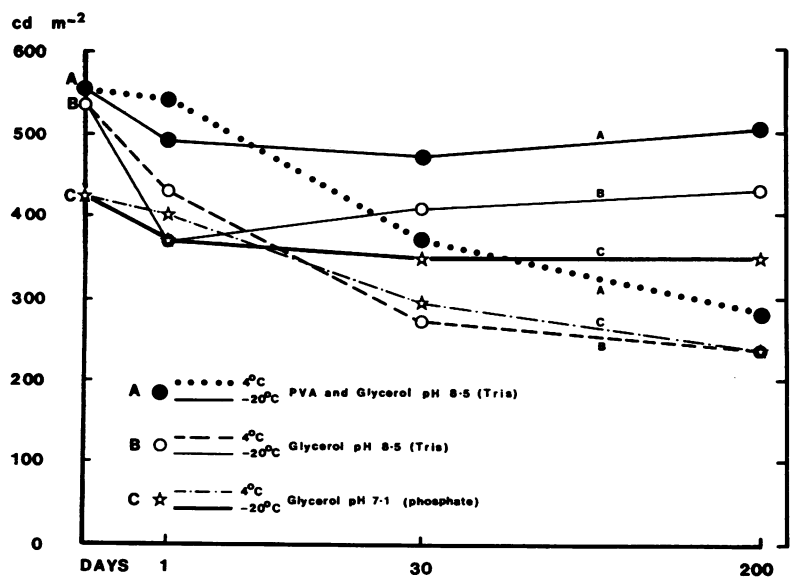

Figure *Comparison of fluorescence emission from* Eschericia coli *in microscopical preparations with different mountants and stored at 4°C and −20°C. Each point plotted represents the mean of 10 readings.*

and fixed by gentle heat. Homologous antibody conjugated with FITC at its routinely used dilution was applied to several smears and left in a moist atmosphere at room temperature for 20 minutes. The smears were washed (with a final rinse in distilled water), dried, and mounted. Excess mountant was removed by applying pressure on the cover glass. To obtain the best optical resolution with mountant A smears must be dry and covered with the minimum amount of reagent.

Slides were examined by means of a Union inverted microscope equipped with a tungsten halogen lamp, a Tiyoda darkfield condenser with toric lens, an all-dielectric interference primary filter with a matching secondary filter (Heimer and Taylor, 1972). Measurements of immunofluorescence emission were made within an hour of mounting as well as after storage at 4°C and at −20°C. The equipment and procedure for making such measurements in terms of candelas per square metre (cd m^{-2}) are described elsewhere (Taylor and Heimer, 1973).

Results

As can be seen from the figure, emission from preparations mounted and stored at −20°C was for mountant A approximately 500 cd m^{-2}, for mountant B 430 cd m^{-2}, and for mountant C 350 cd m^{-2}. All preparations stored at 4°C showed considerable diminution of fluorescence emission. We therefore recommend mountant A, and, that if preparations are to be stored, then −20°C is preferable to 4°C.

We thank Messrs Du Pont for a generous free sample of PVA.

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Symposium on Anticoagulant Control

The International Study Group for the Organization of Anticoagulant Control[1] was formed at an international symposium held on the occasion of the 3rd Mediterranean Congress on Thromboembolism in June 1973. The impetus arose from the fact that at the moment there is no correlation between results of prothrombin time tests used to regulate anticoagulant drug dosage, from hospital to hospital in most parts of the world. The aim was to promote an effective international system of anticoagulant therapy by promoting standardized systems of laboratory control. It is believed that when this is done a meaningful comparison and interpretation of anticoagulant therapy can be made.

The Chairman, Dr L. Poller (Great Britain), stated that the principal aim of standardization of laboratory control of anticoagulants was to make this common form of treatment safer and more effective. An international system of anticoagulant control should be based on the development of national centres for anticoagulant control in each country. He added that WHO had an interest in these developments.

The functions of national anticoagulant control centres could be: (1) to facilitate the provision of standard or reference reagents for their hospitals; (2) to adopt a common system of reporting prothrombin time results. This is essential in order to enable a comparison of the optimum therapeutic range for oral anticoagulant therapy in various cardiovascular diseases; (3) to promote quality control programmes. This is to ensure a correct application of the recommended procedures on a local hospital basis and maintenance of high standards of technical proficiency.

The British system, the only officially adopted national scheme to date, might possibly serve as a model for other countries.

Dr Jean M. Thomson (Great Britain) illustrated the difficulties in anticoagulant

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