

purified prothrombin in the same manner as prothrombin not handled by various laboratory manipulations.

The curves of Fig. 1 also show that the *quantity* of thrombin obtained from purified prothrombin is increased by addition of the plasma factor. As the Ac-globulin concentration is increased, the following changes occur: first, both the rate of activation of the prothrombin and the thrombin yield increase; next, the activation rate shows the predominant increase, but thrombin yield still improves; finally, only activation rate increases, but there is no longer a significant increase in thrombin yield. This sequence of events is the pattern obtained when changes in pH, calcium concentration, NaCl concentration, and other variables are made to interfere with the optimum interaction of prothrombin and thromboplastin (2). Whenever activation rate is appreciably reduced, the eventual yield of thrombin is also reduced. Presumably this is due to the fact that side reactions have time to produce their effect. It has been shown, for example, that partial activation of purified prothrombin yields a substance, very likely thrombin, capable of destroying prothrombin (3).

Through experimental approaches of this kind it has been possible to obtain a preliminary quantitative conception of Ac-globulin concentration and its function. Normal plasma contains the factor in generous quantity. We must, however,

simultaneously consider prothrombin concentration. The current viewpoint is that a bleeding tendency develops when this drops below 10 per cent of normal. It is now apparent that this tendency can be accentuated by a low Ac-globulin concentration. On the other hand, a high concentration of Ac-globulin can compensate for a low prothrombin concentration by forcing rapid production of thrombin and, therefore, quick clotting of blood.

The prothrombin used in this work was prepared by $(\text{NH}_4)_2\text{SO}_4$ fractionation, as described previously (5). It possessed a maximum specific activity of 23,000 units/mg. tyrosine. The Ac-globulin was also purified by methods previously described (6). Prothrombin activity was measured by the two-stage method.

References

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I N T H E L A B O R A T O R Y

Use of Polyvinyl Alcohol¹ to Preserve Fecal Smears for Subsequent Staining

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Fecal samples are often sent for diagnosis to distant laboratories, either preserved with formalin or phenol or in an unpreserved condition. These procedures are adequate for the subsequent identification of any protozoan cysts and helminth forms present, but protozoan trophozoites are almost always destroyed or rendered unrecognizable.

This note describes a method which makes it possible to submit trophozoite material in fixed smears on slides, to be stained and examined when received at the diagnostic laboratory. A fixative is embodied in water-soluble polyvinyl alcohol, which then serves the dual purpose of fixing the fecal smear and forming a temporary mount during shipment.

The mounting medium is prepared by dissolving, in a water bath, 20 grams of powdered Elvanol in the following solution: saturated aqueous solution of mercuric chloride, 130 cc.; 95

per cent alcohol, 60 cc.; glacial acetic acid, 50 cc.; and phenol, 50 cc.

Preparation of smear for shipment. A thin fecal smear is prepared on a clean slide in the usual manner. The smear should not be permitted to dry. It is then covered with a generous amount of the Elvanol solution, using a medicine dropper, and a cover slip applied as in ordinary mounting. In from 2 to 4 hours, depending upon the quantity of solution used and the room temperature, the slide will be dry enough for mailing in any container in which the smears will not be subject to pressure.

Removal of mounting medium prior to staining. The slide is soaked in a 5 per cent aqueous solution of glacial acetic acid at 60°–70° C. until all of the Elvanol film has been dissolved from the smear and the cover slip drops off. This usually takes about 15 minutes. The slide is then rinsed in tap water for 3 minutes and stained in the usual manner with iron-alum hematoxylin. Most organisms will stain as they do following ordinary fixation, except for a tendency to require longer destaining in the differentiating solution.

Preliminary experiments show that protozoan cysts and trophozoites as well as helminth eggs are well preserved by this method. Fecal smears prepared in this manner may be submitted to the laboratory with a reasonable assurance that the intestinal parasites present will be recognizable following staining with iron-alum hematoxylin.

¹ The product used by us is specified as "Elvanol 90-25" (formerly "polyvinyl alcohol, Grade RH-349-A, Type B, medium viscosity"), obtainable from the E. I. du Pont de Nemours & Company, Electrochemicals Department, Niagara Falls, New York.