min A with either rats or chicks, and in the field of nutrition—especially in those cases where fat metabolism is disturbed.

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Natural Formation of Petroleum-like Hydrocarbons From "Oil Shales"

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In many localities throughout the area of the exposed lacustrine Green River facies of the continental Eocene of Colorado, Utah, and Wyoming are exposures of porous and permeable rocks containing a viscous liquid hydrocarbon. This material, soluble in CS_2, CCl_4, ether, and petroleum solvents, has apparently been produced naturally from the enveloping "oil shales."

The best of these porous and permeable beds are several thin, 2 inch to 14 inch layers of volcanic ash (*1*), now largely altered to crystalline analcite and chalcedonic silica. Several of these layers are regionally persistent, but locally there are present from 15 to 36 additional such ash layers ranging from 1/32 inch to 20 inches in thickness. These are intercalated with the organic marlstones or "oil shales" of the Green River beds. Some of these beds, ranging in porosity from 15 to 20 per cent and having a permeability of from 7 to about 30 millidarcys, are enveloped by beds of rich organic marlstone. Standard porosity and permeability tests made on samples of this rich marlstone give results approaching zero, but there is enough permeability along the bedding planes of the material in place for sodium carbonate efflorescence to form on a fresh surface in a month's time.

In areas where there has been no appreciable tectonic activity, these beds of altered volcanic ash are commonly free from all traces of either (1) petroleum-like liquid hydrocarbons soluble in the usual solvents or (2) pyrobituminous material such as is contained in the "oil shales."

However, in local areas of rather moderate folding, such as is encountered near the Grand Hogback in Colorado or along Evacuation Creek in Utah, and in some areas of more gentle dips, some of the less stable, yellowish, amorphous kerogen of the "oil shales" adjacent to these porous analcitic layers has been transformed to a dark brown, waxy, semifluid hydrocarbon. This material fills the pore spaces of the analcitic beds and also the joint cracks of the enveloping "oil shales."

This hydrocarbon is identical with the heavier cuts of shale oils produced from the Green River "oil shales" by usual retorting methods. This may be an intermediate step in the production of gilsonite by inspissation of such hydrocarbons produced by natural (geothermal?) cracking of the pyrobitumens present in the organic marlstone of the Green River lake beds. A substance identical to gilsonite can be produced in the laboratory from "oils" of the type described above.

The above occurrences are offered as field evidence of the existence of such natural "cracking" of pyrobitumens into a liquid hydrocarbon superficially resembling some types of petroleum.

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Plasma Accelerator Factor and Purified Prothrombin Activation[1]

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Recently attention was focused on the presence of a substance in plasma which accelerates the activation of prothrombin (*1*, *6*). Partial purification of this factor has made available a product useful for the study of prothrombin activation (*6*). The newly-recognized factor is a plasma globulin which we refer to as Ac-globulin.

We mentioned in 1938 that partially purified prothrombin is slowly converted to thrombin in the presence of optimum amounts of calcium and thromboplastin (*4*). Such slow activation is illustrated by curve A of Fig. 1. When a small amount of

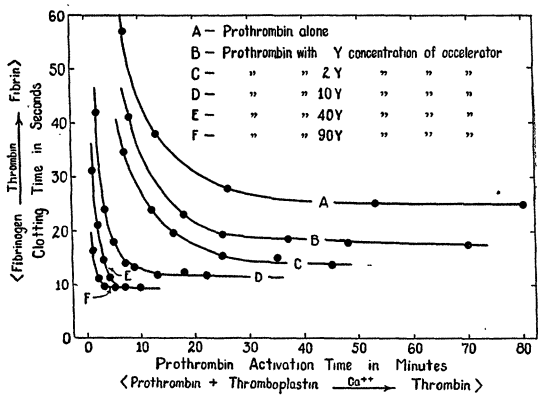

Fig. 1. Activation of purified prothrombin with optimum amount of thromboplastin and calcium. Only Ac-globulin concentration was varied.

Ac-globulin (Y concentration) is first added to the prothrombin, the activation rate of the latter is increased, as shown by curve B. With 2Y, 10Y, and 40Y concentration of Ac-globulin the activation rate is further increased, as illustrated by curves C, D, and E, respectively. Finally, with 90Y concentration of accelerator the activation rate is virtually equal to that of native plasma prothrombin itself (curve F). Heretofore it seemed likely that slow activation of purified prothrombin was the result of damage done to the fragile molecule during the purification procedures. This work shows, however, that it is necessary only to supply Ac-globulin in order to activate

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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 $(NH_4)_2SO_4$ 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.

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IN THE LABORATORY

Use of Polyvinyl Alcohol[1] 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.