one minute.[7] In normal dog blood which is freshly drawn, coagulation is completed in less than 10 seconds, using 1 cc of blood and 0.1 cc of thromboplastin.

Placental thromboplastin is a substance which not only can be used as a local hemostatic, but promises to exert a hemostatic effect when applied parenterally or even intravenously without causing thrombosis. It can be employed in the preparation of thrombin of human origin. It activates thrombin in decalcified plasma, and can be used instead of thrombin in various clinical applications, unless purified fibrinogen solution free from other plasma fractions is preferred.

In preliminary peptone shock experiments, employing Witte's product, kymographic readings demonstrated that the protein compound increases the blood pressure to its level previous to shock, and the dogs recover long before the coagulation time reaches its normal value. *In vitro,* 1 cc samples of peptone blood, in which the control remained incoagulable, were clotted in 1 minute with 0.1 cc thromboplastin. Since in 6 dogs, peptone shock was treated successfully with this placental thromboplastin, it may be of therapeutic value in anaphylactic shock, and perhaps in other forms of shock.

ALFRED LEWIN COPLEY

SCHOOL OF MEDICINE,
UNIVERSITY OF VIRGINIA

RELATIONSHIP BETWEEN PATHOGENICITY AND pH TOLERANCE OF MICROORGANISMS[1]

INVESTIGATIONS on the sterilization of shoes in this laboratory necessitated the microbiological examination of "sterilized" and unsterilized worn shoes to determine the extent to which the causative agents of athlete's foot, the dermatophytes, were removed by the various sterilizing procedures. It soon became apparent that ordinary Sabouraud's dextrose or maltose agar was unsatisfactory for the isolation of dermatophytes when in the presence of the rapidly growing saprophytic fungi commonly found on shoes, floors, etc. (Rhizopus, Aspergillus and Penicillium), for it allowed such saprophytes to grow so rapidly that the agar surface, in most cases, would be covered with the growth of these fungi and thus not allow the slower growing dermatophytes to appear. This same difficulty in isolating dermatophytes was encountered by other workers.[2,3,4]

Studies were undertaken, therefore, to find a method whereby dermatophytes could be selected from mixed cultures and suspensions. Among other factors studied was that of the pH of the culture medium used to isolate the dermatophytes. As acid pH values are used to inhibit bacteria on media for mold cultivation, a series of experiments was made, using Sabouraud's maltose agar adjusted to various acidities and alkalinities and observing the growth of both dermatophytes and saprophytic fungi. As the pH of the medium was increased, the growth of the saprophytic fungi became less dense while the dermatophytes were affected only slightly in comparison. It was soon found that a procedure using Sabouraud's dextrose or maltose agar adjusted to an initial pH of 10.5 with NaOH *immediately before* the plates were poured (hereafter referred to as the *alkaline medium*) and an incubation period of 5½ days at 34° C. gave the maximum inhibition for the saprophytic fungi, while the dermatophytes were inhibited only slightly. Rhizopus and Aspergillus were inhibited almost completely, whereas Penicillium was inhibited much less. Details of this study are to be published elsewhere.[5]

The alkaline medium was tested for its effectiveness in isolating dermatophytes from mixed cultures and from leather suspensions. A marked selective action in favor of the dermatophytes was obtained, while parallel plates using ordinary Sabouraud's dextrose agar were completely covered by growth of the saprophytic fungi. In the form of freshly prepared slants, the alkaline medium can also be used for the isolation of dermatophytes from skin scrapings when saprophytic fungi are present. The full procedure for the isolation of dermatophytes in the presence of saprophytic fungi will be presented more fully elsewhere.[6]

The significant results obtained by increasing the pH of Sabouraud's dextrose of maltose agar showed that the pathogenic fungi, *i.e.,* the dermatophytes, were able by some means to grow at high pH levels while the common saprophytic fungi either did not grow or grew poorly on the alkaline medium. This suggested that pathogenicity might be related to pH tolerance. Tate's work[7] strengthened this suggestion by showing that the pathogenic dermatophytes have an active proteolytic enzyme which is effective in alkaline substrates and which resembles trypsin, while a saprophytic fungus as *Aspergillus niger* does not. This could explain the results obtained with the alka-

[7] A. L. Copley and P. L. Stefko, to be published.

[1] The work described in this paper was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Maryland.

[2] F. W. Weidman, *Penn. Med. Jour.,* 34: 695–701, 1931.

[3] Lee Bonar and Alice D. Dreyer, *Am. Jour. Pub. Health,* 22: 909–926, 1932.

[4] P. A. Neal and C. W. Emmons, *U. S. Pub. Health Service Bull.* No. 246, 1939.

[5] J. M. Leise and L. H. James, *Jr. Lab. and Clin. Med.,* 30: 119–131, 1945.

[6] J. M. Leise and L. H. James, *Arch. Dermat. and Syph.,* in press.

[7] P. Tate, *Biol. Rev.,* 4: 41–75, 1929.

line medium and could also be an explanation of pathogenicity in that pathogens may contain this enzyme while non-pathogens do not or contain it in an attenuated form. Therefore, it would appear possible to differentiate pathogenic from non-pathogenic microorganisms by growth in alkaline broth. This hypothesis was tested by using two strains of *Shigella dysenteriae* and two of *Shigella paradysenteriae* (Flexner), supplied by the National Institute of Health, with one strain of each species being virulent while the other was non-virulent. These bacteria were inoculated into tryptose broth of various pH values with the result that the non-virulent strains were inhibited by alkaline pH values at which the virulent strains were able to grow (a detailed report is in preparation).

The results obtained with the dysentery bacteria in the light of the literature suggest that the pathogenicity-pH tolerance relationship may be due to the presence of a trypsin-like enzyme or enzyme system. If this were shown to be true, it would be possible to use an anti-tryptic agent to change a virulent microorganism into a non-virulent strain and thus aid in treating infectious diseases. The excellent work of Mirsky[8] in inhibiting streptococcus fibrinolysin by trypsin inhibitors and his statement that "It is suggested that the streptococcal fibrinolysin is a protease and that it may be related to trypsin" combines with Tate's studies and our investigations in emphasizing the relationship between pathogenicity and pH tolerance and the importance of a trypsin-like enzyme or enzyme system in pathogenicity.

SUMMARY

An alkaline medium (Sabouraud's dextrose or maltose agar of pH 10.5) was found to be selective for dermatophytes in the presence of the rapidly growing saprophytic fungi and has been used to isolate dermatophytes from mixed cultures and leather. Virulent dysentery bacteria are able to grow in media of alkaline pH values, while non-virulent strains of the same microorganisms are inhibited completely or very greatly. It is strongly indicated that pathogenicity may be related to pH tolerance and it is suggested that this relationship may be explained by the presence of a trypsin-like enzyme or enzyme system, while a loss of pathogenicity is associated with a loss or weakening of this enzyme system.

J. M. LEISE
L. H. JAMES

DEPARTMENT OF BACTERIOLOGY,
UNIVERSITY OF MARYLAND,
COLLEGE PARK, MARYLAND

[8] I. A. Mirsky, SCIENCE, 100: 198–200, 1944.

THE NUTRITIONAL VALUE OF SUNFLOWER SEED MEAL

IN the search for new food materials of significant value in human and animal nutrition scarcely any attempt appears to have been made to critically evaluate sunflower seed. This is surprising because the sunflower (*Helianthus annuus*) is economically important and well known in many parts of the world including the United States and Canada. The production is increasing rapidly. For example, in the season of 1943–44 Argentina's output of sunflower oil amounted to 1,072,000 tons, placing that country second only to Russia as a producer.[1] In 1939–40 the production in Argentina was only 375,000 tons.

The hull-free residue remaining after removal of the oil has been used for livestock and poultry feeding. It is approximately 53 per cent. protein, the biological value of which has been found to be quite low.[2] In all probability this conclusion has been based on analyses of oil-free residue obtained by pressure extraction methods which involved severe heat treatment. Such methods are now known to markedly reduce the biological value of proteins[3] and may be regarded as injurious to certain vitamins. Mitchell *et al.*[3] have recently shown that sunflower seed meal, when produced by a low temperature, solvent extraction process, is in the same class as oats, wheat and barley in regard to the dietary quality of its protein.

Practically nothing can be found in the literature concerning the B complex values of sunflower seed or the product left after removal of the oil. For example, the word sunflower does not even occur in most standard books on nutrition, including the recent "Handbook of Nutrition" of the American Medical Association,[4] although in the latter mention is made of such unusual foods as caviar, crayfish and edible hips of the wild rose.

We have undertaken an investigation of the nutritive properties of the meal left after low temperature, solvent extraction of hulled sunflower seed (Sunrise variety). The marked ability of this product to promote growth and reproduction in rats restricted to purified diets containing it as the only source of B complex vitamins prompts this preliminary note in order that attention might be focused more promptly on this neglected, but promising food.

[1] *Foreign Commerce Weekly*, 17: 37, 1944.

[2] G. Ganchev and I. D. Popov, *Ann. univ. Sofia. V. Faculté agron. sylvicult.*, 14: 209–38, 1936; *Chem. Abstr.*, 31: 3968, 1937.

[3] H. H. Mitchell, T. S. Hamilton and J. R. Beadles, *Jour. Nutr.*, 29: 13–25, 1945.

[4] Council on Foods and Nutrition. "Handbook of Nutrition." American Medical Association, Chicago. 1943.