Satisfactory results obtained by the use of calcium gluconate in prevention and treatment of dogs experimentally intoxicated by DDT suggest that the apparent neurologic symptoms observed are consequent to hypocalcemia, and not due to direct action of DDT upon the central nervous system. It must be emphasized that the six dogs used in the experiments recorded in the present paper are all but one (No. 9) in apparently good physical condition a month after the ending of the experiments.

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STUDIES ON HUMAN PLACENTAL THROMBOPLASTIN IN VITRO AND IN VIVO

AN extract from human placenta exhibiting thromboplastic activity was first described by Sakurai.[1] Eley *et al.*[2] modified his method and prepared a product which did not prove to be highly active on hemophilic blood.[3] Recently Howell[4] prepared a substance from pig lungs of considerable purity and extraordinary potency. He also succeeded in splitting off the active phospholipid. Since human placenta can be obtained so easily, as compared with human lungs from autopsies, I applied Howell's method for that material. However, the resulting protein compound was not as active and not as soluble in alkaline water as compared with the product from human or pig lung. Therefore, a new method has been developed for both the preparation of a protein phospholipid compound and an apparently protein-free substance as tested by the Biuret reaction. In addition human thromboplastin has been prepared recently from saline plasma obtained by diluting blood 10 to 15 times its volume with physiologic saline. Detailed descriptions of the methods of preparation of both the placental and plasmatic thromboplastic substances will be given in a subsequent article.[5]

The new placental thromboplastic substances compare well in potency with Howell's product from pig lung. *In vitro,* in small doses they have caused rapid clotting of hemophilic blood. The materials contain calcium and phosphorus. They are waxy, whitish and wholly soluble in water. Because of a rather high calcium content, they can coagulate decalcified plasma without the addition of calcium chloride. A single placenta yields a large amount of these substances. Greater thromboplastic activity is obtained from the placenta proper than from the cord. In drying the material, for which several methods have been employed, some loss of potency results.

All *in vivo* studies have been made with the protein compound. Following intravenous injection into dogs, the thromboplastin shortens the coagulation time. In large doses there is an initial prolonging effect upon the coagulation time, in some cases the blood becomes incoagulable. However, after several hours the coagulation times return to their original values previous to injection and even have been found to be shortened. This shortening effect lasted between 3 to 5 days in 3 dogs, and for 2 weeks in 2 dogs. The blood-saline coagulation test of Copley and Houlihan[6] has been applied to these studies. This test allows a better measure of the coagulability of native blood than previous methods which tested only the undiluted native blood. The phenomenon of hypercoagulability for a period up to two weeks, after the introduction of human placental thromboplastin into the circulation, can not be explained at present.

Intravenous injections of human placental thromboplastin alone have been made in 15 dogs without general anesthesia. The blood pressure is either slightly lowered temporarily or not at all. There are no apparent ill effects when the thromboplastin is warmed to body temperature and injected slowly. Two dogs which were anesthetized with veterinary nembutal Abbott (0.45 cc per kgm) and thereafter treated intravenously with thromboplastin died in shock. On autopsy one dog exhibited intravascular and intracardial blood coagulation, whereas the other dog was apparently free of coagulation thrombi. This dog died of heart tamponade as a result of an intracardiac injection of adrenalin which was employed in an attempt to alleviate the acute condition of shock. The combination of placental thromboplastin and nembutal appears to produce experimental shock, a phenomenon which merits further investigation. Before the human thromboplastin can be applied *in vivo* by intravenous injections into humans, extensive studies will have to be conducted.

We used this thromboplastin as a local hemostatic in dog surgery resulting in almost instantaneous coagulation of bleeding surfaces. In experiments on coagulation thrombosis in vessel segments of arteries and veins, coagulation was found to occur in less than

[1] K. Sakurai, *Sei-i-kwai M. J.*, 48: 52, 1929. Cited by Eley *et al.* (see footnote 2).
[2] R. C. Eley, A. A. Green and C. F. McKhann, *Jour. Ped.*, 8: 135, 1936.
[3] W. H. Howell. Personal communication, April 7, 1944.
[4] W. H. Howell. Personal communication, May 18, 1943.
[5] A. L. Copley, to be published.
[6] A. L. Copley and R. B. Houlihan, SCIENCE, 100: 505, 1944.

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.

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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.

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[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.