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Staphylococcal Fibrinolysis.*

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The ability of certain pathogenic staphylococci to clot decalcified blood plasma (oxalated or citrated blood) depends upon a mechanism distinct from the activation of prothromblin.[1,2] A recent paper[3] describes the preparation and some properties of a stable staphylocoagulase, together with data to indicate that the co-factor ("activator substance"), which it needs in order to coagulate fibrinogen, can be provided by certain plasma protein fractions, particularly albumins. It seems logical to reserve the term "staphylocoagulase" for the active coagulant and to propose the term *prostaphylocoagulase* for the bacterial product. In tests reported in a previous abstract,[4] the clots formed by the action on *bovine* plasma Fraction-I (fibrinogen) of prostaphylocoagulase plus human serum (or plasma) albumin did not show fibrinolysis for as long as 10 days at 37°C. This is attributable to lack of precursor of the fibrinolytic protease in the bovine plasma product.[5] In the tests of the present communication, *human* plasma Fraction-I is chosen because of its content of pro-enzyme, demonstrated with the aid of streptokinase, as in the previous study.[5] The following experiments prove the ability of the staphylococcal product to act as a kinase activator of the plasma protease precursor and also introduce new evidence that the coagulative and lytic-aiding properties of staphylococci are separable phenomena.

Materials and methods. Previous papers[3,5] give details concerning the reagents: 1. borate buffer (buff.), here used throughout with the addition of 0.4% trisod. citrate (pH: 7.7); 2. H.S.A., human serum albumin; 3. B.F. bovine plasma fraction-I (fibrinogen); 4. H.F., human plasma fraction-I,‡ containing fibrinogen, protease precursor (tryptogen or plasminogen), and co-factor for staphylocoagulase; 5. Strep., streptokinase; 6. Staph. *pro*-staphylocoagulase. In order to secure rapid firm fibrin clots for timing of fibrinolysis, a little thrombin (thr.: the commercial preparation "thrombin topical," 10 units per cc) was added after the other reagents in these tests.

Data. Table I compares the fibrinolytic effects of similarly prepared (1%) extracts of streptokinase (*2*) and (pro)staphylocoagulase (*3*). The *staph.* preparation is considerably slower than the strep. (L.T.—6 min.), but produces complete fibrinolysis in 3 hours. A negligible protease contaminant of the H.F. or thr. (probably the latter) shows up in the control (*1*) as no lysis in 48 hours but about half the clot remaining at the end of 4 days (37°C).

The different thermostability characteristics of the coagulative and lytic effects of the staphylococcal product were studied by incubating a 1% staph. solution in citrated borate buffer and testing at intervals, either

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‡ The human plasma fraction used in this work was prepared from blood collected by the American Red Cross, under contract between the O.S.R.D. and Lederle Laboratories, and supplied through the courtesy of Dr. R. W. Howard, Mr. Frank H. Clarke, and colleagues.

TABLE I.
Effects of Streptokinase and Staphylokinase on Fibrinolysis.

	H.F., 1% 1 cc	Thr., 10u 0.5 cc	Strep., 1% 0.25 cc	Staph., 1% 0.25 cc	Buffer (citrated)	Clot-lysis at 37°C
1	+	+			+	∞ 2 d
2	+	+	+			6 min
3	+	+		+		3 hr

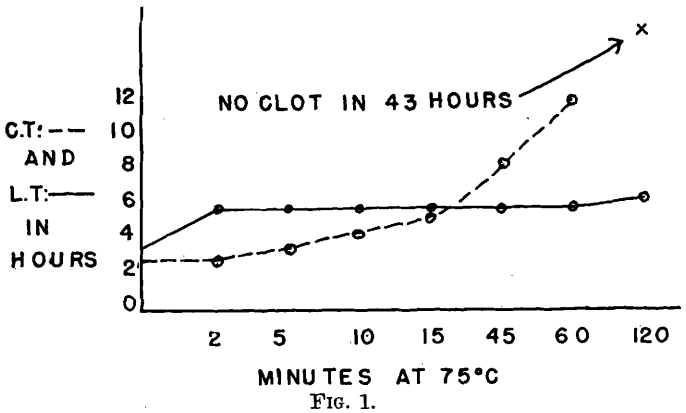

FIG. 1.
Thermostability study of prostaphylocoagulase and staphylokinase. 75°C. C.T.: staphylocoagulation times (at 37°C) for 1 cc B.F. + 0.25 cc staph. + 0.25 cc H.S.A. L.T.: fibrinolysis times (at 37°C) for 1 cc H.F. + 0.25 cc thr. + 0.25 cc staph.

on B.F. + H.S.A. (for staphylocoagulation), or on H.F. + thr. (for fibrinolysis). In a first experiment, at 57°C, the staphylocoagulation times (C.T.) lengthened from 55 minutes at the start to 370 minutes after a 6-hour heating period, whereas the fibrinolysis times (L.T.) remained about the same (3 hours). In a second experiment, the data of which are shown graphically in Fig. 1, incubation was at 75°C, and after 2 hours staphylocoagulation was lost (C.T. > 43 hours), while lysis times were relatively little altered (L.T.: 3-5½ hours).

Conclusions. The data clearly indicate the separability of the coagulative and lytic-aiding properties associated with staphylococci. Other current experiments suggest that the proportions of prostaphylocoagulase and staphylokinase in the alcohol-precipitated product vary with the strain of micro-organism. Since staphylocoagulation is produced by prostaphylocoagulase *plus* a plasma co-factor other than prothrombin (our citration technic gives extra assurance of this), the use of the term "staphylokinase" by Much,[6] to indicate an action like thrombokinase (or thromboplastin), is not correct. We propose the term *prostaphylocoagulase* for this factor, and retain the term *staphylokinase* for the second factor, which acts like streptokinase. In a previous publication[5] we cited recent work which proves that the streptococcal factor does not act as a proteolytic enzyme in its own right (*cf.*[7]), but merely serves as a kinase activator for the precursor of plasma protease (tryptogen or plasminogen). Similarly, the older work (*e.g.*[8]), attributing a protease to staphylococci, must be revised in the light of the data in this present study. We are obviously again dealing with a *tryptokinase*[9]

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or activator of plasma protease. It may be recalled that crystalline pancreatic trypsinogen is activated to trypsin by a mold kinase.[10]

Addendum. In a recent note (*Nature*, 1948, **161**, 559), published after our original communication[4] and received while the present article was in press, C. H. Lack reports confirmation of the staphylo*kinase* factor.

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Oleic Acid Toxicity and Fat Embolism.*

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In view of the paucity of data on the toxicity of fatty acids, experiments with oleic acid were performed. In control tests, olive oil was injected intravenously, and observations were made pertaining to fat embolism.

The hemolytic effects of fatty acids are a well known phenomenon. This report deals with toxic manifestations observed in addition to those of hemolysis. The intravenous injection of fat emulsion was studied by a number of investigators such as Murlin and Riche;[1] Kochne and Mundel;[2] Gordon and Levine;[3] Clark and Brunschwig;[4] Dunham and Brunschwig;[5] McKibbin *et al.*;[6] Shafiroff and Frank;[7] LeVeen;[8] and reportedly by Russian workers who used human fat.

Johnson *et al.*[9-11] have demonstrated increased erythrocyte fragility after the ingestion of large fat meals, due to fatty acids and soaps which enter the blood with the lymph of the thoracic duct.

Dubois *et al.*[12] and Davis[13] demonstrated that serum albumin has considerable affinity for oleic acid, thereby inhibiting the bacteriostatic effects and toxicity of the latter.

For assays of toxicity, 36 normal, starved, unanesthetized, mongrel dogs were used. A few assays were performed on dogs anesthetized with pentobarbital sodium, in which carotid blood pressures were recorded.

The substances used were oleic acid (95% oleic acid and 5% other long chain fatty acids), ethyl, oleate, and sodium oleate; the latter 2 were prepared from the oleic acid. All substances were administered intravenously. The oleic acid and the ethyl oleate and valuable advice were obtained by the kindness of Dr. A. W. Ralston of the Armour Laboratories.

Results. Doses of 0.25 cc of oleic acid had little effect (6 dogs), while doses of 0.5 to 5 cc were toxic (19 dogs). The outstanding

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