pseudogloulin and albumin are devoid of inhibitor, as are the pseudoglobulin of chicken and albumin of horse plasma. Confirming our earlier work,[3] the albumins are found to contain most of the cofactor. Positive results are obtained, however, with euglobulin of horse and pseudoglobulin of horse and chicken (weak). Negative results are found only with guinea-pig pseudoglobulin and chicken euglobulin. Guinea-pig euglobulin could not be tested with certainty owing to fibrinogenolysis. Thrombin interferes with tests on euglobulin in similar experiments with human and some other species of plasma.

Summary. Marked species variations in the staphylocoagulation phenomenon are encountered and can be explained in terms of several factors. Comparing with rabbit plasma as an empirical standard (shortest staphylocoagulation times encountered), the ability to shorten staphylocoagulation time by addition of cofactor (human serum albumin) is evidence for lesser amounts of cofactor in such plasmas as horse and bovine. Cofactor cannot be recovered completely or uniformly in plasma fractions obtained with the classical $(NH_4)_2SO_4$ salting procedures. Nevertheless, the "albumin" is consistently rich in cofactor (for activation of prostaphylocoagulase) and devoid of thrombin (which interferes in tests on some euglobulins).

Excess of inhibitor(s), in part at least directed against active staphylocoagulase as shown by pre-incubation of prostaphylocoagulase with cofactor before adding to plasma (or plasma dilution + fibrinogen) is a feature of some plasmas, notably rat and chicken. Owing to this, the demonstration of cofactor requires plasma fractionation, in these instances.

Fibrinogenolytic and possibly other proteolytic effects may interfere with tests, especially at higher (37°C) temperatures, and are exemplified in guinea-pig and dog plasmas.

The quantitative evaluation of staphylocoagulation times must, therefore, take cognizance of variability not only in the two bacterial factors but, even more significantly, in the various plasma factors on which they operate in the systems devised for testing the staphylocoagulation and proteolytic[9] phenomena. There are marked species differences in these plasma factors.

[9] Gerheim, E. B., and Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., following paper.

Received May 16, 1949. P.S.E.B.M., 1949, **71**.

17156. Species Reactivity to Staphylokinase.*

EARL B. GERHEIM† AND JOHN H. FERGUSON.

From the Department of Physiology, University of North Carolina, Chapel Hill.

The mechanism of staphylococcal fibrinolysis has been shown recently[1,2] to be analogous to the mechanism of streptococcal fibrinolysis,[3] namely, a kinase type of activation by the bacterial product, of plasma or serum proenzyme (protease precursor) to yield the active lytic agent. In an earlier study,[2] of which the present data are an extension, the distinction between the bacterial factor taking part in proteolysis (*staphylokinase*) and that involved in coagulation (*pro-Staphylocoagulase*)[4] was made by means of

* This work was supported, in part, by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

† Predoctorate Research Fellow, U. S. Public Health Service. Present address: Wayne University College of Medicine, Department of Physiology and Pharmacology, Detroit, Mich.

[1] Lack, C. H., *Nature*, 1948, **161**, 559.

[2] Gerheim, E. B., Ferguson, J. H., Travis, B. L., Johnston, C. L., and Boyles, P. W., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 246.

[3] Milstone, H., *J. Immunol.*, 1941, **42**, 109.

[4] Gerheim, E. B., and Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., previous paper.

TABLE I.
Staphylococcal Fibrinolysis and Inhibition by Crystalline Soybean Trypsin-inhibitor, in Plasma Clots of Various Species. 37°C; pH = 7.7 (borate buffer).

Species	I. Staphylokinase	II. Staph. + inhibitor
Dog	25 min.	60 min.
Human	50 min.	165 min.
Guinea pig	60 min.	225 min.
Rabbit	270 min.	No lysis (48 hr)
Ox	No lysis (72 hr)	No lysis (72 hr)
Horse	"	"
Sheep	"	"
Rat	"	"
Chicken	"	"

heat lability experiments. The current study concerns (1) species differences in plasma factors which react with staphylokinase, (2) differences between staphylokinase and streptokinase (a) in these species plasma reactivities and (b) in the rates with which they induce their respective activation of protease precursor.

Reagents. 1. Plasmas were collected from the various animal species by centrifugation of blood received into one-tenth volume of 4% sodium citrate (hydrated); 2. Thrombin was the commercial (Parke, Davis) "Thrombin Topical" diluted to 10-15 units/ml; 3. Fibrinogen was Armour's bovine plasma Fraction-I, which was proenzyme-free[5] inasmuch as no active protease could be detected after treatment with (1) chloroform, (2) bacterial kinases, or (3) "fibrinokinase" of Astrup and Permin;[6] 4. The staphylococcal product, containing both *staphylokinase* and *prostaphylocoagulase* (of no consequence in the presence of added thrombin) was prepared in 1% solution as previously described;[7] 5. Crystalline soybean antiprotease (trypsin-inhibitor), courtesy of Dr. M. Kunitz,[8] was used in 1:10,000 solution; 6. Borate buffer,[5] pH = 7.7, was used as solvent and diluent throughout.

Staphylokinase- and streptokinase-induced fibrinolysis in plasma clots of various species. Confirming earlier workers,[9] we find *streptokinase* to lyse human plasma (fibrin) clots, but unable to induce fibrinolysis in plasma of other animal species.

Staphylokinase is tested in the following systems: I. 0.5 ml citrated plasma + 0.25 ml staphylokinase + 0.25 ml buffer + 0.25 ml thrombin; II. similar mixtures but containing 0.25 ml 1:10,000 crystalline soybean antiprotease instead of buffer. The data given in Table I are representative of a number of experiments.

Staphylokinase does not show the strict species specificity of streptokinase, but does fail to lyse plasma clots (Table I) of ox, horse, sheep, rat, and chicken. Fibrinolysis is obtained, however, with dog, human, guinea-pig, rabbit. Moreover, the respective lysis times are usually in that order both in I. and in II. In the latter series, a weak protease inhibitor is chosen to bring out its relative inhibitory effects and these show a definite correspondence with the initial enzyme potency. Thus, in the case of the weak rabbit protease the inhibitor inhibits fibrinolysis for at least 48 hours, whereas its effect is relatively minor, *i.e.* 60 min. *vs.* 25 min., on the lysis time in dog plasma tests.

In repeated tests on a single species, *e.g.* rabbit plasma, considerable variations in lysis time are encountered, despite the use of the same staphylokinase preparation and carefully standardized test conditions. It may be recalled that Lack[10] consistently failed to acti-

[5] Ferguson, J. H., Travis, B. L., and Gerheim, E. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 285.

[6] Astrup, T., and Permin, P. M., *Nature*, 1947, **159**, 681.

[7] Gerheim, E. B., Ferguson, J. H., and Travis, B. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 525.

[8] Kunitz, M., *J. Gen. Physiol.*, 1946, **29**, 149.

[9] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[10] Lack, C. H., *Brit. J. Exp. Path.*, 1948, **29**, 191.

vate rabbit plasma with his staphylokinase preparations and hence suggested possible plasma inhibitors.

The working out of quantitative methods to estimate and distinguish between plasma differences as to enzyme precursor, on the one hand, and the possible inhibitors, on the other, is beyond the scope of the present communication.

It is, nevertheless, possible to conclude that, in addition to individual plasma variations, there are wider and more significant species variations, clearly illustrated by the data of Table I, which, whatever the ultimate cause, do represent definite species differences in the plasma reactivity of staphylokinase.

Staphylokinase fibrinolysis in human plasma Fraction-I was noted in a previous paper.[2] Clearly, staphylokinase and streptokinase can only be compared on human plasmas or products containing the species reactive proenzyme and equivalent in terms of such still ill-defined factors as the possible antikinase and antiprotease inhibitors.

Rates of activation of proenzyme by staphylokinase and streptokinase. Numerous experiments in our laboratory confirm the conclusion of previous workers[11,12] that streptokinase activates human plasma proenzyme "almost instantaneously" (Kaplan, 1945). In such case, attention is directed away from the fact that the events following the addition of bacterial kinase to plasma clot systems occur in two phases, namely, *first* the activation of proenzyme to lytic agent, and only *secondly* lysis of the substrate (fibrin). Early in our studies of staphylokinase the question was raised whether this activator might not differ from streptokinase in the rapidity of the first phase reaction. The following test system was, therefore, devised to permit independent following of the progress of the activation of proenzyme. Serum (4 ml) was used as a fibrinogen-free source of proenzyme and incubated at 37°C with the staphylococcal product (1 ml). After suitable incubation periods, 0.5 ml of the mixture was added to 1 ml proenzyme-free bovine fibrinogen and immediately clotted with 0.5 ml thrombin. Such experiments were performed on human, rabbit, guinea-pig, and dog sera. Typical results from sera of the last two species are recorded in Fig. 1. It may be seen that the shortest lysis times (optimal protease activity) required an incubation averaging 30-60 minutes. In the experiment with dog serum, the lysis test after 15 seconds incubation was 33 minutes, but reached an optimal of 4 minutes lysis time after incubation for half an hour. Thereafter, the lysis times gradually lengthened, reaching 36 minutes after 4 hours of incubation, obviously denoting instability or inactivation of the enzyme at the 37° temperature. The guinea-pig data are less striking but qualitatively similar.

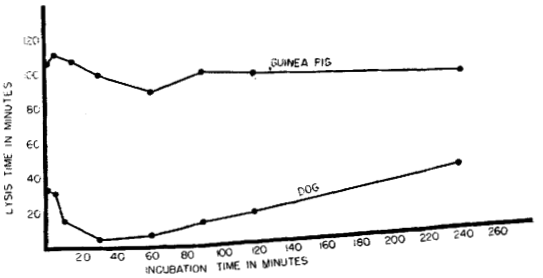

FIG. 1.
Activation of serum proenzyme by staphylokinase.

Summary. Fibrinolytic tests are described which clearly demonstrate differences in the species reactivity of plasmas to the staphylokinase and streptokinase activation of the protease system. Whereas streptokinase acts only on human materials, staphylokinase works on plasma (or serum) of dog, guinea-pig, and rabbit also, but gives negative results with bovine and other species tested. Staphylokinase differs from streptokinase also in requiring an incubation period of many minutes before reaching optimal activation of the serum proenzyme.

Received May 16, 1949. P.S.E.B.M., 1949, **71**.

11 Kaplan, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 40.

12 Christensen, L. T., and MacLeod, C. M., *J. Gen. Physiol.*, 1945, **28**, 559.