17155. Staphylocoagulation in Plasmas of Various Animal Species.*

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Smith and Hale[1] showed that clotting of decalcified plasma by staphylococci takes place in two stages, namely, (1) reaction of the bacterial product (prostaphylocoagulase[2]) with a cofactor or activator-substance in plasma to form the active clotting agent (staphylocoagulase), which (2) then converts fibrinogen into a fibrin which, according to our own observations under the dark-field microscope, appears indistinguishable from ordinary fibrin, despite the fact, which we have indubitably confirmed, that the staphylcoagulation phenomenon can occur in the complete absence of the thrombin mechanism.[2] We have previously reported[3] on the occurrence of cofactor (Tager's[4,5] "coagulase reacting factor") in various blood and tissue products, finding a human serum albumin (see reagents) to be the most satisfactory source material.

In earlier literature on the great variability of the staphylocoagulation phenomenon in plasmas of differing animal species, some suggestions have been made as to lack of cofactor (activator), thermolability of activator substance or of staphylocoagulase, and possible combinations of these and other factors. For instance, it has been stated[1] that fowl and mouse plasmas completely lack activator substance, whereas guinea-pig plasma clots at 20°C but not at 37° owing to low concentration of activator, in consequence of which staphylocoagulase production proceeds at too slow a rate to keep pace with thermal deterioration at the higher temperature (cf. infra). Kaplan and Spink[6] give evidence for a further factor, namely, an inhibitor, demonstrable in the euglobulin fraction of human and guinea-pig plasmas. It is precipitable between 25 and 40% saturation with ammonium sulfate. Tager and Hales[5] also suggest that the coagulase reacting factor may be bound or masked in the plasma of certain species.

The present study is an extension of earlier reports[2,3] and describes experiments in which the plasmas of a number of animal species have been tested with some modified methods and fractioned with classical ammonium sulfate procedures in an attempt to explain more fully the reasons for reduction or absence of staphylocoagulation in the case of certain species.

Reagents. 1. The borate buffer, pH = 7.7, used as solvent and diluent throughout, was citrated to 0.4% $Na_3C_6H_5O_7$, 5½ H_2O; 2. a 1% staphylococcal product, containing ***both*** prostaphylocoagulase and staphylokinase, was prepared as described previously;[3] 3. Fibrinogen was Armour's bovine plasma Fraction-I; 4. Thrombin (10 units/ml) was diluted commercial (Parke, Davis) "Thrombin Topical"; 5. The plasmas of the various species were centrifuged in citrate (0.4%) and, for some tests, fractioned with ammonium sulfate into the classical crude "globulins" and "albumin" as follows: I. 50% saturation with $(NH_4)_2SO_4$ precipitated the globulins which, on dialysis until sulfate-free yielded (a) "euglobulin" precipitate: dissolved in citrated borate buffer to original plasma volume, (b) "pseudoglobulin" supernatant; dialyzed and concentrated *in vacuo* to original plasma volume;

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1 Smith, W., and Hale, J. H., *Brit. J. Exp. Path.*, 1944, **25**, 101.

2 Gerheim, E. B., Ferguson, J. H., and Travis, B. L., *Fed. Proc.*, 1948, **7**, 41.

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

4 Tager, M., *Yale J. Biol. Med.*, 1948, **20**, 369.

5 Tager, M., and Hales, H. B., *J. Immunol.*, 1948, **60**, 1.

6 Kaplan, M. H., and Spink, W. W., *Blood*, 1948, **3**, 573.

TABLE I.
Staphylocoagulability of Various Plasmas, at 37°C and 20°C. Clotting-times with Prostaphylocoagulase; A. with, B. without, Added Cofactor.

Plasma	A. Prostaph. + cofactor		B. Prostaph. + buffer	
	37°C	20°C	37°C	20°C
Rabbit	19 min.	22 min.	18 min.	17 min.
Human	36 min.	54 min.	36 min.	54 min.
Chicken	No clot*	No clot*	No clot*	No clot*
Rat	" "	" "	" "	" "
Guinea pig	240 min.†	324 min.	No clot‡	323 min.
Dog	73 min.	29 min.	180 min.†	28 min.
Horse	100 min.	235 min.	227 min.	228 min.
Bovine	150 min.	660 min.	660 min.	660 min.

* Thrombin added after 24 hr gave solid clot; no fibrinogenolysis.
† Clot not quite solid; fibrinolysis subsequently.
‡ Thrombin added after 24 hr gave no clot; fibrinogenolysis.

II. albumin supernatant from the original globulin precipitation: similarly dialyzed and concentrated to original plasma volume; 6. cofactor (activator) of prostaphylocoagulase was *human* serum albumin prepared as above.

Factors affecting staphylocoagulation in plasmas of various species. Tests were run at 20°and 37°C, timing the coagulation of 0.5 ml plasma with A. 0.25 ml prostaphylocoagulase + 0.25 ml cofactor; B. 0.25 ml prostaphylocoagulase + 0.25 ml buffer. When no clotting was evident after 24 hours, 0.25 ml thrombin was then added to determine whether fibrinogenolysis had occurred incidental to the staphylokinase-protease phenomenon. In order to determine inhibitory effects directed against *active* staphylocoagulase, prostaphylocoagulase and cofactor were incubated at 37° for half an hour before adding the selected plasma. Thus, species differences in staphylocoagulation times could be evaluated, at least semiquantitatively, with respect to (1) low cofactor content; (2) presence of inhibitors (s); 3. interference by protease, or (4) several possible combinations of these factors. The initial data are summarized in Table I, and their analysis is supplemented by the special tests of Tables II and III.

These experiments indicate: 1. Staphylocoagulation times, under carefully controlled test conditions, vary according to species of plasma; 2. Several plasma factors contribute to these species variations, *e.g.* (A) Rabbit, dog, human, and to a lesser extent horse, guinea pig, and bovine, give staphylocoagulation with the bacterial product alone (the citrate precluding any possible thrombic mechanism) and must, therefore, contain cofactor: (B) additional cofactor reduces the coagulation times of horse and bovine plasma, but not to the level of the best clotting times seen with rabbit plasma. Proteolysis is not evident in these species. Hence it is concluded that they are relatively lower in cofactor than the rabbit and also contain some inhibitor: (C) Human plasma gives the same staphylocoagulation times, with or without cofactor, and longer than those for rabbit plasma. In the absence (in these particular experiments) of proteolytic effects, the explanation must be in terms of inhibitor(s): (D) That at least part of the inhibitory effects is against active staphylocoagulase is proved by the method stated. Such inhibitor is very marked in chicken and rat plasmas which do not clot under any of the test conditions, including the addition of preincubated prostaphylocoagulase + cofactor. Since clots occur on the addi-

TABLE II.
Inhibitory Effects of Various Plasma Dilutions; 0.25 ml Added to Mixture*: 1 ml Fibrinogen, 0.5 ml Cofactor, 0.25 ml Prostaphylocoagulase. Clotting-times (min.) at 37°C.

Species	Plasma dilutions				
	1:1	1:8	1:32	1:128	1:256
Chicken	2160	119	44	44	42.5
Horse	318	62	62	40	40
Guinea pig	80	80	64.5	64.5	41

* Control (buffer instead of plasma): 40 min. staphylocoagulation time.

TABLE III.
Tests for Inhibitor and Cofactor.

Species plasma	Inhibitor			Cofactor		
	Euglob.	Pseudoglob.	Alb.	Euglob.	Pseudoglob.	Alb.
Chicken	+	0	+	0	±	+
Horse	±	+	0	+	+	+
Guinea pig	? (lysis)	0	0	? (lysis)	0	+

tion of thrombin after 24 hours, fibrinogenolysis is not significant in these cases: (E) With guinea-pig plasma, our findings are staphylocoagulation at 20°C in a time which is the same whether or not cofactor is added. The last is not a variable, therefore. Nevertheless, at 37°C, clots obtained in the presence of cofactor are not quite solid and undergo fibrinolysis subsequently. Without cofactor, no clot is obtained at 37°C and the 24 hr. test with thrombin is negative, showing that fibrinogenolysis has occurred. Our experiments are a partial confirmation of those of Smith and Hale[1] but complete the explanation in terms of interfering effects of proteolysis induced by the accompanying staphylokinase[7,8] factor in the staphylococcal product, with no need to postulate any instability of the staphylocoagulase. The fact that in guinea-pig plasma staphylocoagulation is faster at 37° than at 20° confirms Kaplan and Spink,[6] but these workers' data are probably more significant as to temperature coefficient since their staphylococcal product would appear to have been deficient in staphylokinase: (F) in dog plasma, proteolysis, at 37°, is also encountered but the staphylokinase interference is less, so that some staphylocoagulation still occurs. That the clotting is slower at the higher temperature, in this case, does indicate greater interference by the proteolysis as the temperature is raised from 20° to 37°C. Moreover, it is slower, at 37°C, when cofactor is not added. Owing to interference by fibrinogenolysis it can be suggested only tentatively that dog plasma has more cofactor than guinea-pig's and less than rabbit's.

[7] 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.

[8] Lack, C. H., *Nature*, 1948, **61**, 559.

Inhibitory effects of various plasmas on staphylocoagulation. In order to investigate further the inhibitory phenomena, the special tests summarized in Table II were performed on plasmas selected from chicken, horse, guinea-pig, *i.e.* representative, respectively, of 1. antistaphylocoagulase, 2. inhibitor accompanying lesser amount of cofactor, 3. lesser cofactor with accompanying proteolysis. Tests were performed at 37° C on mixtures of fibrinogen (bovine, 1 ml), cofactor (human albumin, 0.5 ml), and prostaphylocoagulase (0.25 ml). To this 0.25 ml of various plasma dilutions was added. As compared with a 40 minutes staphylocoagulation time in the control (buffer instead of plasma), definite inhibitory effects were apparent in all cases and lessened on dilution. The inhibitor is highest in undiluted chicken plasma. Proteolytic effects did not significantly interfere when guinea-pig plasma was tested under these conditions.

Cofactor and inhibitor of staphylocoagulation in various plasma fractions : The euglobulin, pseudoglobulin and albumin fractions of the plasmas of the above three species were tested: 1. in a repetition of the foregoing demonstration of inhibitor, using only the full strength plasma fraction, however; 2. in a similar test system but omitting the human albumin (cofactor) in order to detect the presence of cofactor in the various plasma fractions. The data are given, qualitatively, in Table III.

We have noted that no fraction yields an inhibitor concentration comparable to that of the original plasma and, in fact, the total inhibition by all fractions does not equal that of the whole original plasma. Owing to proteolysis, it is not possible to be certain of the inhibitory effect of guinea-pig euglobulin. Its

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.

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17156. Species Reactivity to Staphylokinase.*

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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

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