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Fibrinolytic Properties of Filtrates of *Clostridium histolyticum* in Normal Saline and Dog Serum.

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Within the past few years, considerable study has been devoted to filtrates of *Clostridium histolyticum* grown on various media. This organism, when cultured anaerobically on a protein-rich medium, yields a filtrate displaying marked proteolytic and deaminizing effects^{1, 2} upon complex protein substances such as fibrin, casein, coagulated egg albumin and tumor tissue.³ Studies made on this and other proteinases suggest that they do not have any particular specificity for any single protein complex present in the substrate. According to Diehl⁴ the enzyme or enzyme groups constituting the proteinase are formed to correspond to different amino acids in the medium and will attack these whether combined or free. The activity of the proteinase of *Cl. histolyticum* depends on pH. However, various optima ranging from pH 7.0² to pH 8.0-9.0⁵ have been reported. Some workers have found more acid optimum values.⁶ Filtrates of *Cl. histolyticum* are also believed to show increased proteolytic activity in the presence of sulphhydryl compounds and various metallic ions.^{1, 2, 7}

Connell and his associates⁸ have used parenteral injections of filtrates of the organism grown on specific tumor tissue in the hope that such material might possess sufficient enzymatic specificity to attack tumor tissue *in vivo*. Their results have been neither thoroughly confirmed nor refuted.^{8, 9, 10} The original purpose of the short series of experiments to follow was to determine the fibrinolytic

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² Weil, L., and Koehalaty, W., *Biochem. J.*, 1937, **31**, 1255.

³ Connell, H. C., *Canad. Med. Assn. J.*, 1935, **33**, 364.

⁴ Diehl, H. S., *J. Infect. Dis.*, 1919, **23**, 347.

⁵ Walburn, L. E., and Reyman, G. C., *J. Path. and Bact.*, 1934, **39**, 669.

⁶ Blanc, J., and Pozerski, E., *Comp. rend. Soc. Biol.*, 1920, **83**, 1369.

⁷ Stevenson, Marjory, *Bacterial Metabolism*, Longmans, Green & Co., New York, 1930.

⁸ Pommerenke, W. T., *J. A. M. A.*, 1936, **106**, 1654.

⁹ Rusch, H. P., and Preston, W. S., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 581.

¹⁰ Moppett, W., and Goldsworthy, N. E., *Med. J. Australia*, 1936, **23**, 754.

activity of filtrates of *Cl. histolyticum* grown on various media with particular reference to their possible use in clinical conditions associated with intravascular fibrin deposition and clotting, for example subacute bacterial endocarditis.

The culture of *Cl. histolyticum* used by Weil and Kochalaty² (American Type Culture Collection No. 4972) was obtained for these experiments. The organism was cultured anaerobically on various media for 4 days at 37°C, after which the material was passed through a Zeitz filter. The filtrate was added in varying amounts to normal saline (pH 6.7) on the one hand, and dog serum (pH 7.2) on the other to produce 10 cc of mixture in which the filtrate dilution was 2.5, 5.0, 10, 20, and 30% respectively. In each test tube a block of sterile dog fibrin weighing approximately 0.5 g was immersed. The test tubes were shaken several times daily and kept at a temperature of 37°C. All procedures were performed with sterile technic. Preliminary experiments showed that the addition of cysteine monohydrochloride as activator was without appreciable effect. The index of fibrinolytic activity (F) was arbitrarily determined by the formula,

$$F = \frac{1}{T \times C}$$

T = time in days for complete fibrin liquefaction and C = percentage concentration of filtrate producing liquefaction.

TABLE I.

Culture Medium	Normal Saline			Dog Serum		
	C	T	F	C	T	F
Neopeptone broth	2.5	1	.40	20	3	.017
Horse meat broth	2.5	1	.40	5	7	.029
Clotted dog blood*	2.5	1	.40	5	5	.040
Dog serum	2.5	1	.40	20	3	.017
Particulate fibrin masses in normal saline	2.5	3	.13	No effect		

F = fibrinolytic index.

T = time in days for complete liquefaction of fibrin.

C = percentage concentration of filtrate producing liquefaction.

*Liquefied by growth.

From Table I it appears that in several rich protein media, the *Cl. histolyticum* produces filtrates with high fibrinolytic potency. It is also evident that this potency is tremendously decreased in the presence of dog serum. When grown on a simple medium of fibrin in saline, a filtrate of low potency was produced. Controls containing no filtrate did not produce fibrin liquefaction.

The antienzymatic power of serum has long been recognized and

its effect on various enzymes studied, for example, trypsin,^{4, 11} leucoprotease,¹² lipase, etc. Blanc and Pozerski⁶ found decreased power of gelatin liquefaction of *Cl. histolyticum* filtrates in the presence of raw serum albumin. Other enzymes have been studied more extensively in this respect.¹³ Furthermore, the result of repeated injection of such materials into animals is to increase the antienzymatic effect of the serum^{14, 15} and to produce antibodies.¹⁶ The exact nature of the substance involved in the antienzymatic mechanism is not yet completely understood. It seems reasonable to assume from our experience and that of others with *in vitro* experiments, that such fibrinolytic filtrates would not be effective *in vivo*.

Summary. 1. Filtrates of *Cl. histolyticum* grown on various protein-rich media possess a high fibrinolytic potency, whether sulphhydryl activator be present or not. 2. This fibrinolytic potency is greatly depressed in the presence of dog serum. 3. It is probable that the use of filtrates of *Cl. histolyticum* as a fibrinolytic agent in dissolving fibrin deposits *in vivo* would not be feasible due to the antienzymatic properties of blood serum.

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Heparin and the Blood Coagulation Mechanism.*

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The efficacy of heparin as an anticoagulant was first observed by Howell and Holt.¹ Later Howell² demonstrated the presence of heparin in the blood and described a method for its purification.

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¹² Opie, E. L., *J. Exp. Med.*, 1906, **8**, 536.

¹³ Opie, E. L., *Physiol. Rev.*, 1922, **2**, 552.

¹⁴ Achalme, P., *Ann. de l'Inst. Pasteur*, 1901, **15**, 737.

¹⁵ Bergmann, von, und Bamberg, *Berl. klin. Wochenschr.*, 1908, **45**, 1396.

¹⁶ Connell, H. C., *Bull. Hendry-Connell Research Foundation*, 1938, **3**, 3.

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² Howell, W. H., *Am. J. Physiol.*, 1924, **71**, 553.