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Activation of Proplasmin by a Tissue Fraction.

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The proteolytic enzyme plasmin (fibrinolysin) is found in normal blood plasma in an inactive form, called proplasmin. Activation can be produced by treatment with chloroform, ether and some other organic solvents,[1] as well as by streptokinase.[2] The active form of the enzyme is occasionally found in the blood of certain types of patients,[3] but the mechanism of activation in these cases and whether there exists a physiological mechanism of activation of the enzyme in the normal organism, are at present unknown.

The evidence presented in this report indicates that an activator is present in tissue extracts which could constitute the normally occurring activator of the enzyme.

The lungs of the freshly killed adult rats were removed and forced through a 1 mm mesh. The tissue pulp was ground in a mortar, suspended in 30% sucrose solution[4] and then submitted to differential centrifugation in the cold. Tissue fractions and supernatants were obtained and labeled according to the nomenclature of Claude.[5] For testing the material, the substrate used was 0.2% fibrinogen solution in 0.01 M phosphate buffer in saline at pH 7.2, kept frozen at −10°C, prepared by the method of Ware *et al.*[6] The tissue fraction was mixed with either human blood serum or a globulin solution prepared by precipitating human blood plasma at 45% saturation of ammonium sulfate at pH 7, dissolving the precipitate in a volume of saline equal to ¼ the volume of plasma, and dialyzing against saline for 3 days. The serum or the globulin solution served as a source of proplasmin. Fibrinogen was then added and the mixture was clotted by the addition of thrombin (Lederle clotting globulin) on a glass rod. Time of dissolution of the clot at 37°C was noted.

Table I shows the activity of the different fractions from lung tissue. The presence of inhibitory action was evident in tubes 2 and 6, but no inhibitory action was noted on increasing the amount of microsome fraction (tubes 7 and 8). Control experiments showed that the tissue fractions without addition of globulin solution or the globulin solution alone had no dissolving action on the fibrin clot within the time of observation (2 hours) but occasionally the tissue fractions without addition of globulin exhibited such activity over a period of 16 to 24 hours. This may have been due to slight contamination of the fibrinogen preparation with proplasmin.

Fresh blood serum could be used instead of the globulin solution as a source of proplasmin with essentially similar results.

Conclusion and summary. The data indicate that on mixing tissue pulp from the lungs of freshly killed adult rats with blood serum or a fraction of blood serum containing proplasmin, fibrinolytic activity appeared in the mixture. This was presumably due to activation of proplasmin of serum by a tissue kinase present in the lung tissue. The kinase activity was found to be concentrated in the microsome fraction of the tissue, while the inhibitory activity was in the supernate. The

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1 Tagnon, H. J., Davidson, C. S., and Taylor, F. H. L., *J. Clin. Invest.*, 1942, **21**, 525.

2 Christensen, L. R., *J. Gen. Physiol.*, 1945, **28**, 363.

3 Tagnon, H. J., Levenson, S. M., Davidson, C. S., and Taylor, F. H. L., *Amer. J. Med. Sc.*, 1946, **88**, 211.

4 Hogeboom, G. H., Schneider, W. C., Palade, G. E., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 320.

5 Claude, A., *J. Exp. Med.*, 1946, **84**, 61.

6 Ware, A. G., Guest, M. M., and Seegers, W. H., *Arch. Bioch.*, 1948, **18**, 231.

TABLE I.
Action of Tissue Fractions on Proplasmin.†

Tube No.	Reagents in cc.* Total extr.	Mitochondria	Supernate 1	Microsomes	Supernate 2	Diss. of clot (min.)
1	.1					15
2	.3					27
3		.1				30
4		.3				35
5			.1			16
6			.3			26
7				.1		11
8				.3		9
9					.1	120+
10					.3	120+

† All fractions were suspended in a volume equal to that of the original extract.
* Fibrinogen 0.2 cc + globulin solution 0.1 cc + 0.01M phosphate buffer 0.3 cc, thrombin (on glass rod) added to each tube. Temp. 37°C; pH 7.2.
Control experiments: 1. Same as in table with omission of globulin solution: no dissolution in 2 hr. 2. Same as in table with omission of fractions of tissue: no dissolution in 2 hr.

system described here may be identical with that proposed by Astrup and Permin.[7] It is known also that the microsome fraction of lung tissue contains most of the thromboplastic activity of the total tissue extract,[5] although this should not be construed as indicating an identity between the kinase and thromboplastin.

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

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Exoerythrocytic Stages of *Plasmodium cynomolgi* in the *Macaca mulatta*.*

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The exoerythrocytic cycle from sporozoite to erythrocytic parasite has now been demonstrated in 4 species of avian malaria.[1-5] The exoerythrocytic stages, called cryptozoites and metacryptozoites by Huff and Coulston,[4] are widely distributed throughout the tissues of the avian host and develop in cells of the lymphoid-macrophage system and in endothelium.

Recently, Shortt and Garnham,[6,7] and Hawking[8] described pre-erythrocytic stages in the livers of monkeys (*Macaca mulatta*) in-

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[1] Huff, C. G., and Coulston, F., *Bi-monthly Report, Comm. Med. Res., Office of Scientific Research and Development*, 1943, No. 12, June 1.

[2] Reichenow, E., and Mudrow, L., *Deutsche tropenmed. Z.*, 1943, **47**, 289.

[3] Coulston, F., and Huff, C. G., *J. Infect. Dis.*, 1947, **80**, 209.

[4] Huff, C. G., and Coulston, F., *J. Infect. Dis.*, 1944, **75**, 231.

[5] Huff, C. G., Coulston, F., Laird, R. L., and Porter, F. J., *J. Infect. Dis.*, 1947, **81**, 7.

[6] Shortt, H. E., and Garnham, P. C. C., *Nature*, London, 1948, **161**, 126.

[7] Shortt, H. E., and Garnham, P. C. C., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1948, **41**, 785.

[8] Hawking, F., *Nature, London*, 1948, **161**, 175.