STUDIES ON THE PNEUMONIC EXUDATE.

I. Effect of Preservation, Temperature, Dialysis, and Salt Concentration on the Enzyme in the Pneumonic Lung.

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In a previous report one of us[1] reported the demonstration in cellular material obtained from the pneumonic lung of a proteolytic enzyme active in eroding the surface of Löffler's blood serum at hydrogen ion concentrations of 7.3 to 6.7 and inactive at more acid concentrations. Evidence was also brought forward of the presence in the cellular material of a proteolytic enzyme splitting peptone to amino-acid nitrogen. This enzyme was operative at hydrogen ion concentrations from 8 to 4.8 but most active at 6.3 or 5.2.

In this report we present certain further observations on the conditions influencing the action of the enzyme on Löffler's blood serum.

Maintenance of Activity of the Enzyme under Preservation.—The enzyme capable of eroding Löffler's blood serum still remains active after preservation in the ice box mixed with chloroform and toluene for a period of 18 months.

Effect of Temperature on the Enzyme.—The enzyme is active at incubator temperature. It is slightly active at room temperature and inactive in the ice chest. Its activity still persists after heating at 56° or at 65°C. for 1 hour. After heating at 75°C. for 1 hour the enzyme is inactive.

Effect of Dialysis.—To test the penetration of a celloidin sac, the following experiment was performed. 5 cc. of cellular material obtained from the pneumonic lung were placed in a celloidin sac previously sterilized by submersion in 60 per cent alcohol for 1 hour. The cellular material was allowed to dialyze against 5 cc. of sterile normal saline solution[2] for 1 hour. One loopful of the saline solution

[1] Lord, F. T., *J. Exp. Med.*, 1919, xxx, 379.

[2] By normal saline in this series is meant 0.85 per cent.

outside the sac and one loopful of the cellular material from within the sac were placed on the surface of Löffler's blood serum. The saline solution showed no erosion of the medium while the cellular material from within the sac showed erosion.

Effect of Salt Concentration on the Enzyme.—Material obtained from a pneumonic lung was washed through gauze with normal saline solution and the washed suspension centrifuged. The heavy grayish cellular suspension thus obtained was diluted with about 3 cc. of saline solution to make about 12 cc. of total suspension. Equal parts (0.5 cc.) of this cellular suspension and solutions of sodium chloride of different normality were mixed together as follows:

Tube No.	Normality.[2]	Sodium chloride.	Cellular suspension.
		cc.	*cc.*
1	N	0.5	0.5
2	2 × N	0.5	0.5
3	4 × N	0.5	0.5
4	8 × N	0.5	0.5
5	16 × N	0.5	0.5
6	32 × N	0.5	0.5

One loopful of each mixture was placed on the surface of Löffler's blood serum and incubated over night. All showed proteolysis. Increased concentration of sodium chloride therefore does not seem to inhibit the enzymatic action of the material on Löffler's blood serum.

CONCLUSION.

1. The enzyme present in the pneumonic lung exudate still remains active after preservation for 18 months.
2. The enzyme is active at incubator temperature before and after heating to 65°C. for 1 hour. It is slightly active at room temperature and inactive after heating at 75°C. for 1 hour.
3. Dialysis of the enzyme is not demonstrable.
4. Activity persists when the enzyme is mixed with concentrations of sodium chloride varying from normal to thirty-two times normal.

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