STUDIES ON THE PNEUMONIC EXUDATE.

II. The Presence of Enzyme and Antienzyme in the Pneumonic Lung. Local Ferment-Antiferment Balance.

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It has long been evident that the exudate in lobar pneumonia disappears by absorption and the investigations of Müller[1] and Simon[2] suggest that autolysis is responsible. Simon demonstrated autodigestion in gray to a greater degree than in red hepatization. This was later confirmed by Silvestrini[3] and by Flexner.[4] The work of Opie[5] and others has demonstrated that the autolytic process is with probability to be regarded as due to ferments set free from the polynuclear cells.

An important relation is known to obtain between ferments set free from cells and antiferment contained in serum. Thus Opie,[5] in his investigation of the capacity for proteolytic digestion of pleural exudates artificially produced in dogs and rabbits, showed that the serum of an inflammatory exudate has the power of inhibiting the action of proteolytic ferments contained in the leucocytes.

Jobling, Petersen, and Eggstein[6] investigating the blood of patients with pneumonia found that the crisis is usually accompanied by a decrease in the serum antiferment and an increase in ferment. According to their view, ferment, probably derived from the tissue

[1] Müller, Fr., *Verhandl. schweizerischen Naturforschenden. Ges. Basel*, 1901, xiii, 308.

[2] Simon, O., *Deutsch. Arch. klin. Med.*, 1901, lxx, 604.

[3] Silvestrini, R., *Biochem. Centr.*, 1903, i, 713.

[4] Flexner, S., *Tr. Assn. Am. Phys.*, 1903, xviii, 359.

[5] Opie, E. L., *J. Exp. Med.*, 1905, vii, 316; 1906, viii, 410.

[6] Jobling, J. W., Petersen, W., and Eggstein, A. A., *J. Exp. Med.*, 1915, xxii, 568.

cells of the body in general, may be mobilized and brought to bear on the diseased organ, the active autolysis which begins at the time of crisis depending on an altered relation between the ferment-antiferment balance. As they point out, the isolation of the involved region from the general circulation favors autolysis by hindering the access of blood serum with its great concentration of antiferment. The following experiments represent an attempt to determine more clearly the source of the ferment and the application of the ferment-antiferment balance to the pneumonic exudate.

We have usually found that the purulent sputum obtained from patients in the later stages of lobar pneumonia erodes the surface of Löffler's blood serum. The exudate at autopsy from pneumonic lungs in the later stages of the disease commonly also produces erosion. Inasmuch as the culture tubes almost invariably show bacterial growth the question may be raised whether the action of living bacteria or their products may not be responsible for the erosion. In our experience, however, the bacteria found in the pneumonic lung do not alone cause erosion of the medium, and we have in one instance obtained from the pneumonic lung in the stage of purulent softening sterile material capable of eroding blood serum slants, suggesting that the action of living bacteria is not concerned in the process. Such an experience does not exclude the possibility that an enzyme derived from the dissolution of pneumococci may not be responsible.

Tests for the presence of an enzyme derived from dissolved pneumococci and capable of eroding Löffler's blood serum were performed as follows: The bacterial growth of Type I pneumococcus obtained after incubation over night of a liter flask of glucose broth was centrifuged. The sediment thus obtained was added to 8 cc. of sterile bile and incubated for 3 hours. The bile was then centrifuged at high speed. Films from the small amount of sediment showed large numbers of Gram-negative and eroded remains of pneumococci. The bile in which the pneumococci had been dissolved caused no erosion of blood serum. No evidence is presented therefore suggesting that an enzyme derived from the pneumococcus is the cause of the erosion.

The following experience indicates that a substance inhibiting proteolysis exists in early pneumonic areas and can be washed out of the exudate. Mashed material obtained from a pneumonic lung in the stage of red hepatization when placed on the surface of Löffler's blood serum failed to erode the medium. The same material after washing five or six times with normal saline solution produced erosion of the medium, indicating that the inhibiting substance was thus removed or the enzyme set free.

The following experiment shows that normal human serum in certain concentrations will inhibit the proteolytic action of the pneumonic exudate and in lower dilution still allow the proteolytic action of the exudate to take place. It furnishes strong support to the belief that the inhibiting substance removed from the lung in the previous experiment was an excess of serum containing antiferment. Mixtures were made of cellular material obtained from the mash of a pneumonic lung in the stage of gray hepatization and normal human serum to produce varying proportions of cellular material and serum as follows:

10 cc. of the lung mash (preserved with toluene and chloroform) were ground several minutes in a mortar. 7 cc. were drawn off with a capillary pipette, placed in a centrifuge tube, and spun down for 1 minute at low speed. The supernatant fluid was pipetted off and spun down at high speed. This final sediment was washed twice with saline solution and recentrifuged at No. 7 plug on the rheostat for 7 minutes. The colorless and comparatively clear supernatant fluid was pipetted away and 0.5 cc. of "cells" remained. These were made up to the desired dilutions with saline solution. Serum was obtained from fresh human blood and dilutions were made with saline solution. Precautions to maintain sterility were used throughout. The dilutions of "cells" to make the cellular suspension and the dilutions of serum are indicated in Table I. 5 drops of the cellular suspension and 5 drops of the serum were placed in sterile tubes and kept at incubator temperature for 15 minutes. Then 1 drop of the mixtures was placed on Löffler's blood serum and the presence or absence of erosion noted after 18 to 24 hours in the incubator. No growth of bacteria was observed on the culture media.

As shown in Table I, the presence or absence of er ion depends on the proportion of cellular material to serum in the ixtures and not upon the dilution of the cellular material, since in the three groups (Tubes 1 to 4, 5 to 8, and 9 to 12) the dilution of the cellular material was the same in each tube of any one group. A fairly definite relation obtains between the proportion of cellular material to serum and the production of erosion. No erosion was noted when the ratio was less than one part of cellular material to four parts

TABLE I.

Ferment-Antiferment Balance in the Erosion of Löffler's Blood Serum.

Tube No.	Dilution.		Amount of cellular suspension.	Dilution.		Amount of serum.	Final dilution.		Ratio of "cells" to serum.	Erosion.
	"Cells."	Saline solution.		Serum.	Saline solution.		"Cells."	Serum.		
			gtt.			*gtt.*				
1	1 part.	4 parts.	5	1 part.	0	5	1:10	1:2	1:5.0	0
2	1 "	4 "	5	1 "	1 part.	5	1:10	1:4	1:2.5	+
3	1 "	4 "	5	1 "	4 parts.	5	1:10	1:10	1:1.0	++
4	1 "	4 "	5	1 "	6.5 "	5	1:10	1:15	1:0.67	+++
5	1 "	9 "	5	1 "	0	5	1:20	1:2	1:10.0	0
5	1 "	9 "	5	1 "	1 part.	5	1:20	1:4	1:5.0	0
7	1 "	9 "	5	1 "	4 parts.	5	1:20	1:10	1:2.0	+
8	1 "	9 "	5	1 "	6.5 "	5	1:20	1:15	1:1.33	++
9	1 "	19 "	5	1 "	0	5	1:40	1:2	1:20.0	0
10	1 "	19 "	5	1 "	1 part.	5	1:40	1:4	1:10.0	0
11	1 "	19 "	5	1 "	4 parts.	5	1:40	1:10	1:4.0	0
12	1 "	19 "	5	1 "	6.5 "	5	1:40	1:15	1:2.66	+(?)

of serum. Questionable erosion resulted when there was one part of cells to 2.66 parts of serum, and enzymatic action was demonstrable when the cellular material exceeded this amount in the ratio.

DISCUSSION.

The autolysis of the pneumonic exudate may be regarded as a local chemical process capable of its peculiar evolution because of the isolation of the alveolar exudate from the general circulation by the limiting alveolar wall. The disintegration of the cellular

alveolar exudate is the chief source of ferments and the activity of the ferments depends on the amount of serum in the affected region. As the inflammatory process advances the cellular elements become more numerous and the serum less abundant. The cellular ferments increase with the continued disintegration of the cells and the inhibiting action of the serum ceases in the alveoli, allowing proteolysis to take place. The experiment cited above suggests that antienzymatic action of the serum terminates when the cells reach a greater concentration than one part of cells to approximately three parts of serum. The framework of the lung may be assumed to be supplied with sufficient blood to maintain the necessary excess of antiferment. By such a mechanism as this the lung framework is spared permitting resolution of the exudate and on the part of the lung restoration to normal. It is not surprising also that at times the mechanism should fail in consequence of an improper local ferment-antiferment balance. Too great an impairment of the circulation and an excess of ferment are probably concerned in the disintegration of the pulmonary framework in postpneumonic abscess. Too free a supply of blood with its content of antiferment may be regarded as of importance in delayed resolution.

CONCLUSIONS.

The purulent sputum obtained during life and the exudate at autopsy from the later stages of lobar pneumonia commonly erode the surface of Löffler's blood serum. Cellular material obtained from the pneumonic lung in an early stage of lobar pneumonia failed to erode the surface until washed with normal saline solution. Mixtures of washed pneumonic cellular material and normal human serum fail to erode Löffler's blood serum when the amount of cellular material is less than one part of cells to approximately three parts of serum. Erosion occurs when the cellular material exceeds this amount in the ratio.