SPECIAL ARTICLES

ALCOHOLIC FERMENTATION BY FUSARIA JUICE OBTAINED WITH A WET CRUSHING MILL

THE study of certain aspects of the mode of action of cell-free yeast juices from the point of view of colloid chemistry was introduced several years ago,[1,2,3] and led, among other developments, to the experimentally established rule that enzymes in juices and within the cell may change their activity in accordance with the degree of dispersion of the accompanying carriers. Miss M. G. Macfarlane[4] presents an impressive, though presumably far from complete list of differences in behavior between the living yeast cell and the so-called cell-free extracts in regard to the mechanism of alcoholic fermentation. The fermenting cell-free juices generally used (prepared according to Buchner, Lebedew or Lipmann) necessarily exhibit partial or complete suppression of activities or losses of a single or several members of the enzyme systems involved. This accounts for the well-known fact that the harmonious order of the living cell and the phase sequence of enzyme action within it become disorganized due, *e.g.*, to dilution, disruption of carrier systems because of possible denaturation, proteolysis, etc.

A cell system which exhibits, beyond doubt, a measurable phosphorylation and subsequent dephosphorylation of carbohydrates during fermentation is that of the Fusaria. It was deemed justified, therefore, to attempt to investigate an artificial enzyme system from *Fusarium lini Bolley* extracted with the aid of the Booth-Green wet crushing mill.[5] This juice, due to the manner of its preparation, could, if found to be at all active, be compared to a large extent with the juice obtained long ago by Dixon and Atkins,[6] but scarcely investigated by them.

After applying a slight mechanical improvement to the mill, we obtained a juice which can be described as follows. It is opaque; its color varies from light yellow to reddish-brown, according to the distribution of the pigment present.[7] Its pH, prepared with water, is in the neighborhood of 5.7 (the internal cell pH of *Fusarium lini Bolley* lies between 6.0 and 6.1[8]); its relative viscosity measured at 25.3° is 1.168, the determination on the juice having been made a few days after preservation at 6°.

The measurable esterifying capacity of the juice at 28°, as determined by the method of Fiske and Subbarow,[9] amounts under our experimental conditions to about 35 to 40 per cent. of the phosphorylation in the living cell system. In some cases this appears to have been preceded by a dephosphorylation of the organic phosphate donators originally present in the living Fusaria cells.[10] The esterification brought about by the quantity of inorganic phosphate present amounted to about 3 to 4 per cent. The quantity of CO_2 obtained by the action of the zymases compared favorably with that of the living system.

Judging from the data available at present, and taking into consideration the fact that the crushing of the cell makes uniform the highly different pH values of closely organized cell particles,[11] the cell-free Fusaria juice obtained with the wet crushing mill seems to have furnished us so far with an enzyme system which does not exhibit deficiencies or distortions of kind or magnitude as compared with the usual maceration juices obtained from yeasts.

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[1] F. F. Nord, *Trans. Faraday Soc.*, 26: 760, 1930.
[2] *Ibid.*, SCIENCE, 75: 54, 1932.
[3] *Ibid.*, *Nature*, 135: 1001, 1935.
[4] M. G. Macfarlane, *Biochem. Jour.*, 33: 574, 1939.
[5] Booth and Green, *Biochem. Jour.*, 32: 855, 1938.
[6] Dixon and Atkins, *Sci. Proc. Royal Dublin Soc.*, 14 [N. S.]: 1, 1913.
[7] Nord, Hofstetter and Dammann, *Biochem. Zeits.*, 293: 235, 1937.
[8] S. Mahdihassan, *Biochem. Zeits.*, 226: 203, 1930.
[9] Fiske and Subbarow, *Jour. Biol. Chem.*, 66: 375, 1925.
[10] Nord, Hofstetter and Dammann, *Biochem. Zeits.*, 293: 252, 1937.
[11] J. Spek, *Ergebn. Enzymforschung*, 6: 20, 1937.

THE ACTION OF TYPE-SPECIFIC ANTIBODY UPON THE PULMONARY LESION OF EXPERIMENTAL PNEUMOCOCCAL PNEUMONIA[1]

ALTHOUGH the effectiveness of type-specific antiserum in the treatment of pneumococcal pneumonia is well established, the action of antibody upon the pulmonary lesion is not clearly understood. Considerable evidence[2,3,4] has been advanced supporting the view that antibody can not penetrate areas of consolidation within the lung. Recently Kempf and Nungester[5] have studied the penetration of antipneumococcal immune bodies into pneumonic lesions produced experimentally in rats. Using both horse and rabbit antiserum, they were unable to demonstrate the presence of antibody in the lungs following intravenous treatment. They concluded that, even were it found to penetrate the consolidated area, the antibody could not be expected to accumulate in sufficient concentration to neutralize the pneumococcal polysaccharide present in the alveoli.

[1] Preliminary report.
[2] B. S. Kline and M. C. Winternitz, *Jour. Exp. Med.*, 21: 311, 1915.
[3] T. T. Wang and C. M. Van Allen, *Proc. Soc. Exp. Biol. and Med.*, 30: 814, 1933.
[4] J. P. Fox, *Jour. Immunol.*, 31: 7, 1936.
[5] A. H. Kempf and W. J. Nungester, *Jour. Infect. Dis.*, 65: 1, 1939.

Nye and Harris,[6] studying human autopsy material, have reached the same conclusion.

The purpose of this paper is to report preliminary data which demonstrate that antipneumococcal immune bodies not only enter the pneumonic lesion but do so in sufficient amounts to limit its spread. The antibody is shown to cause agglutination of the pneumococci within the alveoli and to promote rapid phagocytosis of the organisms by the cells of the pneumonic exudate.

Experimental lobar pneumonia was produced in white rats by inoculation of the left main bronchus with encapsulated Type I pneumococci[7] suspended in mucin. The method of Nungester and Jourdonais,[8] modified to increase the severity of the disease, was used in all experiments.[9] The pneumonia produced was uniformly fatal, killing all of 38 untreated rats in less than 5 days. Half of the animals died within 48 hours. In only 4 rats was the lesion confined to the left lung, and all but 1 developed pleurisy, pericarditis, or both. All had bacteriemia at the time of death.

The pathogenesis of the pulmonary lesion was studied by examining the lungs of 35 rats killed at various intervals following inoculation. The pneumonic process was seen to spread rapidly until nearly the entire left lung was involved in 36 hours. Frequent blood cultures showed invasion of the blood stream in roughly half of the animals after 12 hours and in over 90 per cent. at the end of the first day. The lungs of each rat were fixed in Zenker-formol solution according to the method of Loosli,[10] and sagittal sections of the single-lobed left lung were stained by the Gram-Weigert technique. The most striking feature of the spreading lesion was the presence of the edema zone[11,12] at the advancing margin. The pneumococci were seen to be most numerous in this region and histological examination of the lungs at intervals from 12 to 36 hours revealed that the pneumococci spread into normal alveoli principally by way of the edema fluid.

The action of type-specific antibody upon the advancing lesion was observed in rats treated 2, 6, 12 and 18 hours after inoculation. One cc (6,000 units) of concentrated antipneumococcal rabbit serum[13] was injected into the femoral vein of each animal in a single dose.[14] The rats were killed in groups of 3 at 1, 6, 18, 42, 96 and 168 hours after treatment; the lungs were fixed and stained by the methods already described. No deaths due to pneumonia occurred among the 76 treated animals, in spite of the fact that many had already developed bacteriemia at the time of treatment. The bacteriemia cleared following therapy and autopsy findings indicated that the pulmonary lesion had been promptly arrested by the serum. The margin of the consolidated area was seen in the gross to become sharply demarcated 6 to 18 hours after treatment, in striking contrast to the irregular hemorrhagic border characteristic of an advancing lesion. Microscopic examination showed pronounced agglutination of the pneumococci, particularly in the edema zone, within an hour. Organisms with swollen capsules were noted frequently. Following agglutination of the pneumococci, the pneumonia failed to spread as evidenced by narrowing of the edema zone at 6 hours and its disappearance after 18 to 42 hours. The agglutination of the organisms in the edema zone appeared to prevent them from invading new alveoli and thus apparently allowed leucocytes to overtake them at the margin of the lesion. Marked phagocytosis of pneumococci by the polymorphonuclear leucocytes was noted at 6 hours and few, if any, organisms could be found, either extracellularly or intracellularly, after 96 hours. The macrophage reaction[15] became prominent in 4 days, and at the end of one week there was extensive clearing of the exudate.

Conclusions

Type-specific immune bodies contained in antipneumococcal rabbit serum, when administered intravenously in sufficient quantity, enter the pneumonic lesions of rats with experimental pneumococcal pneumonia. The antibody agglutinates the pneumococci free in the alveoli and thus apparently stops the spread of the pneumonic process. Clumped cocci can be seen throughout the involved area, but particularly in the edema zone at the advancing margin of the lesion. The presence of antibody within the alveoli is likewise revealed by the "Quellung" phenomenon and by a marked increase in the phagocytosis of organisms by the cells of the alveolar exudate.

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[6] R. N. Nye and A. H. Harris, 2nd, *Am. Jour. Path.*, 13: 749, 1937.

[7] A5 strain, kindly supplied by Dr. O. H. Robertson.

[8] W. J. Nungester and L. F. Jourdonais, *Jour. Infect. Dis.*, 59: 258, 1936.

[9] Details of the method will be described in a later publication.

[10] C. G. Loosli, *Arch. Path.*, 24: 743, 1937.

[11] O. H. Robertson, L. T. Coggeshall and E. E. Terrell, *Jour. Clin. Invest.*, 12: 467, 1933.

[12] F. D. Gunn and W. J. Nungester, *Arch. Path.*, 21: 813, 1936.

[13] Obtained through the courtesy of Dr. W. G. Malcolm of the Lederle Laboratories.

[14] Subsequently, smaller amounts of antibody were used with similar results. As little as 120 units was found to be effective in rats treated 12 hours after inoculation.

[15] O. H. Robertson and C. G. Loosli, *Jour. Exp. Med.*, 67: 575, 1938.

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