9363 P

Effect of a Heat-Resistant Enzyme Upon the Antigenicity of Pneumococci.

RENÉ J. DUBOS AND COLIN M. MACLEOD. (Introduced by O. T. Avery.)

From the Hospital of the Rockefeller Institute, New York.

Rabbits immunized by the intracutaneous injection of heat-killed encapsulated pneumococci fail to develop the type-specific carbohydrate antibodies which result from the intravenous injection of the same antigen.[1] An attempt was made to follow the fate of pneumococci injected into the skin, in the hope of determining the cause of this lack of type-specific antigenic response.

Heat-killed cells of pneumococcus Type I were injected at several sites into the skin of a rabbit. The injected areas were excised at different intervals of time and films made from the tissue fragments were stained by the Gram technic. There was, of course, a pronounced polymorphonuclear infiltration at the site of injection. The pneumococci were seen to undergo a process of extracellular digestion which began within 24 hours after injection and was completed in 4 to 5 days; many bacteria became Gram negative before being engulfed by the leucocytes. These observations suggested that leucocytes produce ferments capable of attacking heat-killed pneumococci.

To demonstrate the existence of these enzymes, a polymorphonuclear exudate was obtained by injecting aleuronate into the pleural cavity of a rabbit. The washed cells extracted with N/10 HCl yielded a soluble principle which has the property of rendering pneumococci Gram negative. Active extracts, with similar properties, were obtained from the organs—especially the liver, pancreas, spleen, and lungs—of several animal species. The same enzyme was also prepared from the pleural exudate from a tuberculous patient, who developed empyema following secondary infection with *H. influenzæ*.

The active enzyme present in these extracts is heat-resistant, especially at slightly acid reactions. Its rate of activity upon pneumococci increases with temperature up to 75°C.; the range of pH activity lies between pH 5.5 and pH 9.5. The enzyme appears to be a protein which is rapidly destroyed by pepsin but is completely resistant to trypsin and chymotrypsin.

[1] Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 441.

As stated above, the purified enzymic preparation renders heat-killed pneumococci Gram negative, whether the bacteria are R or S variants, and irrespective of type-derivation. The cells, however, do not undergo dissolution but retain their characteristic morphology; the turbidity of the bacterial suspension is also very little altered.

Following the repeated intravenous injection of heat-killed pneumococci Type I which have been digested with the enzyme, no precipitins for the capsular polysaccharide appear in the serum, while these invariably occur if the untreated cells are injected. It appears, therefore, that the enzyme has the property of inactivating the capsular antigen of virulent pneumococci.

The enzyme, however, does not decompose the capsular polysaccharide itself. In fact of all the soluble substrates tested, yeast nucleic acid* was the only one to be attacked by the purified preparations. A description of this reaction is presented elsewhere.[2]

It is interesting to point out that some preparations of crystalline trypsin† and chymotrypsin† were found to exhibit a small measure of activity against the Gram positive structure of pneumococci and against yeast nucleic acid. After repeated recrystallizations, however, the proteolytic enzymes no longer have any action upon either of these substrates.

9364

Differentiation of Blood Groups in Dogs Based on Antigenic Complexes Present in the Erythrocytes.*

DANIEL MELNICK† AND GEORGE R. COWGILL.

From the Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven.

When dogs are subjected to prolonged, intensive plasmapheresis, the hematocrit values tend to fall to anemia levels in spite of the

* The authors are indebted to Dr. P. A. Levene for a sample of yeast nucleic acid.

2 Dubos, R., *Science*, 1937. In press.

† The authors are indebted to Doctors J. H. Northrop and Dr. M. Kunitz for supplying several samples of crystalline trypsin and chymotrypsin.

* The expenses of this investigation were defrayed by a grant from the Research Fund, Yale University School of Medicine, and by an Alexander Brown Coxe Fellowship Grant.

† Alexander Brown Coxe Fellow, 1936-37.