II. A NOTE ON THE SIGNIFICANCE OF GELATIN FOR BACTERIAL GROWTH.

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IN a study of the effect of various substances present in meat extracts on growth and peroxide formation in pneumococcal cultures it was found that no growth of the strains used occurred in peptone water, and only occasionally in bouillon. The addition of small amounts of Coignet's "Gold Label" gelatin to either of these media always resulted in abundant growth. Previously, Robertson, Sia and Woo [1924] had found that the period of survival of pneumococcus can be considerably increased if 0.1 % gelatin be added to suspensions of these organisms in water or saline. Gelatin purified by Loeb's method also had a beneficial effect on the pneumococcus. The results of the writer's work suggest that 0.1 % commercial gelatin would have some nutritive value for the organism. This is also the view of Meleney and Zau [1924] deduced from the effect of small amounts of gelatin on haemolytic streptococci in saline suspensions.

The growth of pneumococcus in Parke Davis peptone water containing various percentages of gelatin was determined. 3 % gelatin proved to be most suitable. Higher concentrations, up to 16 % gelatin, caused an increase in the duration of the lag period of growth, but viable organisms were present after 3 weeks' incubation. In peptone water containing less than 3 % gelatin, decreases in the amount and in the period of growth were observed.

A 20 % solution of "Gold Label" gelatin, adjusted to p_H 7.8 was autoclaved for periods varying from 20 minutes to 20 hours. No difference in the amount of growth of pneumococcus could be observed in peptone water containing 5 % gelatin from these autoclaved solutions. In the absence of peptone and on the addition of 0.5 % sodium chloride, growth was only obtained in a 15 % gelatin solution autoclaved for 20 hours, and, to a lesser extent, in the sample autoclaved for 18 hours. Growth was also possible in concentrations of 5–10 % of the sample autoclaved for the longest period.

A sample of the commercial gelatin was purified by washing at p_H 4.7 according to the method of Loeb [1922], hydrochloric acid being used instead of acetic acid. All the washings were concentrated *in vacuo*, most of the acid being removed in this process. It was found that growth of the pneumococcus occurred in peptone water containing either the concentrated washings or

the washed gelatin. A mixture of these fractions, in approximately the same proportion as in the original sample of gelatin, had an effect on growth similar to that of the untreated gelatin. Growth was also possible in bouillon on the addition of 2 % purified gelatin.

A portion of the concentrated washings was dialysed in a parchment shell against distilled water at 2°. The concentrated dialysate was found to promote growth of pneumococcus in 2 % washed gelatin-peptone media whilst the dialysed solution had no effect. The ash of "Gold Label" gelatin and of the washings used above have an influence on the growth of the organism similar to that of the dialysate. Furthermore, it was repeatedly found that the addition of 4 % of a Ringer-Locke solution to peptone water allows growth of the pneumococcus to take place in peptone water when washed gelatin is present.

It may be stated in view of the above observations and of other evidence obtained in the course of this investigation that relatively complex protein decomposition products are required for the growth of the pneumococcus. These may be supplied by bacteriological peptones (preferably with a low free amino-acid content) or by hydrolysed gelatin. Secondly, the addition of purified gelatin will allow or will increase growth of pneumococcus in peptone water or in bouillon. Thirdly, in "Gold Label" gelatin there are important impurities which are dialysable and not destroyed by incineration. Ringer-Locke solution may be substituted for these substances.

The following medium was prepared in order to determine whether the recognition of the three foregoing conditions is sufficient to satisfy the growth requirements of the pneumococcus. A 10 % solution of purified gelatin was partially hydrolysed by autoclaving for 24 hours at 120°. Growth of pneumococcus was obtained in a portion of this medium containing the ash of the washings. The media were inoculated with a small amount (0.1 to 2.5 cc. medium) of a dilute suspension of the organism in saline.

Preliminary experiments have been carried out to test the effect of gelatin on the growth of some of the more delicate bacteria other than pneumococcus. Two strains of *B. diphtheriae* were found to grow in a 3 % gelatin-peptone medium and not in either bouillon or peptone water. Better growth of a strain of meningococcus and of two strains of streptococcus was obtained in the gelatin-peptone than in bouillon or peptone water, but the strains of gonococcus examined would not grow in any of these media.

It should be pointed out that the direct assimilation of gelatin by pneumococcus is improbable as this organism does not liquefy gelatin, nor has it, according to Avery and Cullen [1920], any enzyme capable of hydrolysing proteins. Of the many physical properties of gelatin solutions which may affect the growth of bacteria, the decreased solubility and rate of diffusion of oxygen are of particular interest in the case of pneumococcus. Gillespie [1913] has recorded the fact that an inoculum must contain more pneumococci to produce growth in a liquid than on a solid medium. It may be that, before

the initiation of growth of the pneumococcus, a certain minimum value of oxygen tension is essential. This condition, however, is probably realised by the reducing action of the organisms if the area inoculated be sufficiently limited by physical conditions such as obtain in gelatin solutions.

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