liquor, was thoroughly mixed with jelly-coat solution. After about one minute the suspension was centrifuged and the supernatant tested for its sperm-agglutinating ability. In case this was still present, additional sperm was added and the operation repeated until the jelly-coat solution was inactive against spermatozoa.

It is suggested that those groups in the jelly-coat which agglutinate spermatozoa are the ones responsible for the reaction with "antifertilizin". We are trying to identify these groups. It is known that U.V. irradiation abolishes the sperm-agglutinating ability of jelly-coat solutions[1]. We have found that U.V. irradiation abolishes the precipitation reaction between jelly-coat solution and "antifertilizin", and this may be a further indication of similar or identical groups involved in both reactions.

Evidence has been given for the presence in the cortical layer of the sea-urchin egg of a glycoprotein similar to or identical with the jelly-coat substance[2]. It has also been suggested that this glycoprotein may act as an inhibitor whose removal may be the first step in the activation of the egg[3]. Furthermore the cortical layer of the *Arbacia* egg has been shown to contain also ribonucleic acid[4].

The results presented in this paper give evidence of the formation of complexes between jelly-coat substance and cytoplasmic nucleoproteins. A similar complex may well be present in the cortical layer of the unfertilized sea-urchin egg, the fertilizing spermatozoon being instrumental in breaking it down and thereby liberating the nucleoprotein.

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Department of Physiology, Zoological Station, Naples, February 8, 1952.

Zusammenfassung

Bei der Reaktion zwischen einer Lösung von Gallerthüllensubstanz und Extrakt von Seeigeleiern *in vitro* werden u.a. Nukleoproteine ausgefällt. Die Reaktion wird durch Behandlung der Gallerthüllensubstanz mit Spermatozoen oder durch Ultraviolettbestrahlung aufgehoben.

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Action of Capsular Polysaccharide and a Polygalacturonate on the Development and the Virulence of Pneumococcus Type III

It is well known that the virulence of pneumococcus is especially due to the presence of a capsular polysaccharide, specific for every type (S.S.S.). In the case of pneumococcus III, the particular virulence is, according to WOOD and SMITH[1], related to the presence of an especial "slime layer" of the capsula which interfers notably with surface phagocytosis. The slime layer can be made visible by means of fresh staining with methylene blue which stains it metachromatically. This metachromasia is caused, according to REEVES and GOEBEL[1], by a high molecular polymer of 4 β glucuronosidoglucose.

We have known for some time that some strains of virulent pneumococcus, if cultivated on ordinary media, lose both the capsula and the virulence, passing from the form "S" to the form "R". Among the several ways of restoring the lost virulence, we may mention the simultaneous injection of cultures of capsulated and virulent pneumococci killed by heat (GRIFFITH). This procedure leads to a new virulence and also to the transformation of the type. The substance which provokes this transformation seems, according to the studies of MAC CARTHY AVERY and MAC LEOD[2], to be a desoxyribonucleid acid.

A certain action upon the degree of virulence of the germ seems also to be exercised by the same S.S.S. MORRIS and KERR[3] have in researches on immunity noted an augmentation of the pathogenic action on mice of pneumococcus III sensibilized with the homologous S.S.S.

In these preliminary experiments, I have made virulent some pneumococcus III strains by addition of a small quantity of S.S.S. III to the culture media. I have made use of three strains of pneumococcus III, of different origin, which were already virulent for the mouse, I have indicated them respectively by the abbrevations: BG42; ML47; TR49. The BG42 strain had been preserved by passing through broth T; the ML47 strain by drying *in vacuo*; the TR49 on desiccated spleen of a infected mouse. All these strains were found by biological test to have lost their virulence completely; and they showed on selective media (blood-agar, serum-agar, etc.) colonies of pneumococcus in the "R" form which when inoculated intraperitoneally into small white mice, even in large quantity, never caused the death of animal.

Nor was it possible to restore the virulence of the strains after several passing in mice: while the injection together with the Proteus which provoked a putrid peritonitis resulting in the death of the animals, did not allow me to separate a pneumococcus which was virulent in successive inoculations.

However the colonies, which have developed on culture media in the presence of blood or serum, have shown themselves to be of "R" form and not made up of encapsulated elements.

The addition of 0·5 mg of S.S.S. III[4] to every tube of broth with addition of ascitic fluid made it possible starting from the original strains, to obtain the pneumococci in capsulate form. Inoculation into mice confirmed the virulence with a high percentage of deaths from septicemia. In some case I also had the production of inflammatory pulmonary lesion with hepatization.

In order to control the behaviour of the capsular slime layer of the virulent pneumococcus III, I used fresh staining with alkaline methylene blue up to 1%. With such a method the pneumococci without capsules are immediately stained an intense blue, due to rapid diffusion of the stain into the body of the microbe, while the slime-covered organisms take the stain more slowly and feebly, making it possible to assume a more or less evident metachromasia. Generally a greater degree of metachromasia corresponds to a higher virulence of the microbe.

In order to ensure if the virulence which I obtained with addition to culture medium of S.S.S. III, should

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Table I

Strain	Number of inoculated mice	Dead after hours					Living after 72 hours
		12	24	36	48	72	
BG42	10	—	2	3	2	—	3
ML47	5	—	—	2	1	1	1
TR49	10	1	2	3	3	—	1
Total	25	1	4	8	6	1	5

be considered a very specific phenomenon, or if it were possible to reproduce it by putting the germ into conditions of utilising substances chemically analogous to capsular S.S.S., I tasted the activity of *pectines*, which are very similar in chemical structure to the S.S.S. of the pneumococcus. For the introduction of pectines as culture media, already suggested by FUNCK[1] as a substitute for agar, a practical application has not yet been found. I then prepared a pectine culture medium, adding to the ordinary agar up to 2% (fused in the water-bath), a colloidal solution of *polygalacturonate* of Ca to 6%, in the measure of 2 cm³ for every tube of agar, and then allowed it to solidify.

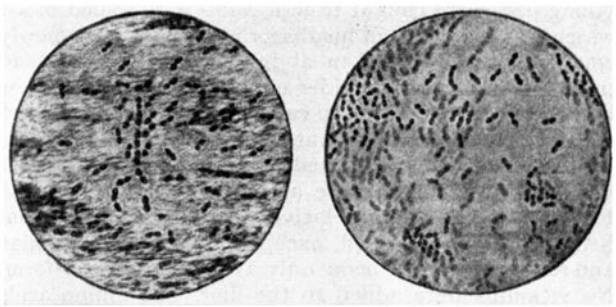

Fig.1.–Pneumococci type III methylene blue fresh staining. On the left: broth culture with ascitic fluid and specific polysaccharide. On the right: ordinary agar with polygalacturonate culture.

Such a medium looks homogeneous and transparent; the avirulent strains of pneumococcus stroke-inoculated produced many colonies of "S" form, with a tendency to flow together in abundant growth of creamy aspect. The growth appeared maximally after 48 hours incubation, and its luxuriant aspect compared well with that obtained with other culture media used before. The pneumococci in such a culture showed capsula and presented metachromatic reaction. When injected into mice, it produced death by sepsis in many animals.

A certain number of mice, however, were able to survive the infection, probably due to some individual resistence. If a certain quantity of polygalacturonate of Ca in solution up to 6% (0·5 or 1 cm³ for a mouse of 20–30 g) is injected some days later into these surviving mice, it is possible to determine in some of them death due to pneumococcal septicemia, while the injection of the same quantity of polygalacturonate is tolerated by normal mice without apparent disturbances.

The polygalacturonate therefore seems to exert an action on the pneumococcus's III virulence, whether *in vitro* or *in vivo*.

Luxuriant and virulent growths are obtained equally if, instead of simple nutrient agar for preparation of the polygalacturonate-medium, agar with addition of serum or ascitic fluid is used.

A frequent characteristic of pneumococci growths in polygalacturonate-agar is a loss of the agar-transparency corresponding to the colonies, so that after removal of these, an exact impression of the growth may persist in the medium. The phenomenon is probably in connection with phenomena of an oxydative nature. It has long been known by MAC LEOD and GORDON[1], PAULI[2] and PLATT[3], that the pneumococcus during its increase is able to give rise to peroxide of hydrogen. The opacity of the agar is reproduceable by oxidation with H_2O_2 and can be modified by addition of ascorbic acid. Pneumococci type III, cultivated on ascorbic acid-polygalacturonate-agar, have shown a marked metachromasia to methylene blue. Such a metachromasia is observed only in colonies which are first developed, and it seems to decrease noticeably with further development of the growth.

This phenomenon, which is worthy of further investigation, could be brought into relationship with the observation of ROBERTSON, ROPES and BAUER[4] on the degrading action of pectine by the H_2O_2-ascorbic acid system. This on the other hand, may exert a destructive action upon various pneumococci capsules, so that we could hypothetically assume that at first the pneumococcus without capsule utilizes more easily the products of degradation of pectine for the synthesis of its own capsular S.S.S., and is afterwards exposed in to turn to the degrading activity of the above-mentioned oxide-reductive system.

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Table II

Strain	Number of inoculated mice	Dead after hours					Living after 72 hours
		12	24	36	48	72	
BG42	10	—	1	3	2	—	4
ML47	10	—	—	2	4	—	4
TR49	10	—	2	1	3	1	3
Total	30	—	3	6	9	1	11

Conclusion: (1) The addition of S.S.S. to the culture media can restore the virulent character of strains of pneumococcus III which have long ago lost their pathogenetic power.

(2) The addition of polygalacturonate of Ca to nutrient agar, with or without addition of serum or ascitic fluid, gives an excellent medium of culture for the pneumococcus, which increases there luxuriantly in "S" form, capsulated and virulent.

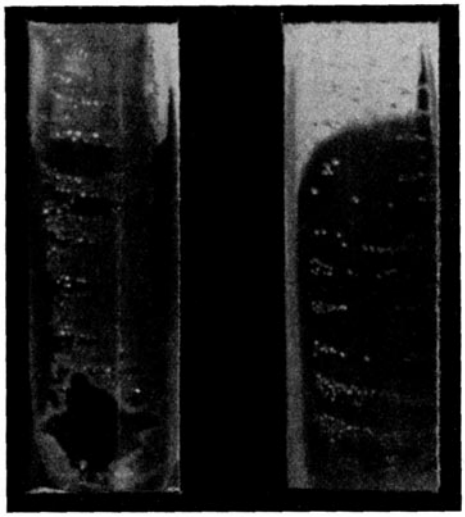

Fig. 2.–Culture of pneumococcus III on polygalacturonate-agar (on the left) compared with normal culture on serum-agar.

(3) The injection of polygalacturonate into mice infected by pneumococcus III, increased its pathogenic power, while it did not produce evident toxic effects in normal mice.

(4) The growth of pneumococcus III on polygalacturonate-agar may show a modification of transparency of the medium, presumably in relation to phenomena of oxydative nature.

(5) The addition of ascorbic acid to the polygalacturonate-agar appears primarily to facilitate, and then to inhibit the development of elements with capsular metachromasia.

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Zusammenfassung

Durch Züchtung nicht-pathogener Pneumokokkenstämme in Gegenwart von spezifischen Polysacchariden wurden diese für weisse Mäuse virulent. Ein besonders günstiges Medium für die Entwicklung von Pneumokokken wurde bei der Prüfung von Pektinstoffen auf Nährböden gefunden, wenn man nämlich den gewöhnlichen Agarkulturröhrchen 2 cm^3 einer kolloidalen Lösung von 6prozentigem Polygalakturonat des Ca zusetzte. Auf diesem Nährboden entwickeln sich die Pneumokokken (III) in reichem Masse, sowohl in eingekapselter wie auch in virulenter Form.

Amino Acid Requirements for Growth of the Honeybee (*Apis mellifica* L.) [1]

In a previous communication [2] it was shown that a normal growth of the young worker honeybee, determined by the estimation of dry weight and nitrogen content, could be obtained on a semi-synthetic diet of sucrose and casein at a temperature of 30°C. The natural diet of sucrose and bee bread (pollen) was used for comparison. Now we have obtained this growth of the newly emerged imago on a totally synthetic diet. The casein of the former investigation being replaced by a mixture of 17 amino acids, based on the composition of casein [3]. The dicarboxylic acids of casein, i.e. glutamic- and aspartic acid, were omitted from the mixture after it had been shown that a casein hydrolysate from which these amino acids had been quantitatively removed with the ion exchange resin Amberlite I.R. 4*B*, could replace the casein. (A full account of these findings will be published elsewhere.) From the amino acid mixture each component was omitted in turn and the dry weight and nitrogen content of the bees was determined at 7 days intervals. In this way we were able to investigate the significance of each of the amino acids for the protein anabolism of the young honeybee.

Material and methods.–In September–November 1951 young bees were caught from a comb with sealed brood which was placed in an incubator at ± 33°C. The newly emerged bees were taken at intervals of 8 hours and transmitted into Liebefelder experimental cages. These little cages, with 50 bees in each, were kept at 30°C and constantly supplied with water and with the experimental diet. The latter consisted of sucrose, tap water and mixtures of 17 or 16 pure amino acids (Hoffmann-La Roche [4]) in the same relative amounts as in casein. *l*-Amino acids were used, except methionine, threonine and serine, the latter being only available in the *dl*-form. No vitamins were added to the diet. The amino acids were fed at such a level that the normal nitrogen content of an adult bee could be obtained in about 14 days. This occurs if the amino acid mixture amounts to 2·5% of the dry sugar. The amino-acids are added to the sugar-water mixture (4:1) and the whole is heated for some minutes to dissolve the sugar. After cooling the mixture is stirred with a glass rod. In this way a very finely crystallised sugar candy is obtained in which the amino acids are dissolved or divided homogeneously.

Dry weights and nitrogen contents were determined of newly emerged bees, and of bees after having been fed for 7 and 14 days on the experimental diets. For each determination a sample of 5 bees was used, after removing the digestive tract, with honey stomach and excretory organs included.

The dry weight was determined by keeping the samples for 3 days in an incubator at 80°C. Nitrogen determinations were performed according to KJELDAHL, with sulphuric acid, potassium sulphate and selenium mixture (WIENINGER) for destruction, and 0·1 *n* solutions of sulphuric acid and sodium hydroxyde for titration. For further details see [5] and [6].

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