five observations: two where the abscesses were free of both amoebae and bacteria, and three where definite evidence of spontaneous healing was present.

As a result of the inoculation of the amoebae and bacteria into the liver, we have failed to produce abscesses in some instances while in other instances we have produced abscesses which ranged all the way from purely bacterial ones with no amoebae in them to those which contained only amoebae. In those in which only bacteria were present, the amoebae had probably been killed off or crowded out by the growth and activity of the bacteria, while in those where the activities of the bacteria were partly checked by the cat, it was possible for both amoebae and bacteria to live together as they do in the lumen of the intestine or in culture media. Whenever amoebae were present in an abscess, regardless of whether bacteria were present or not, there was no membrane or wall of granulation tissue at the edge or periphery of the abscess. This fact made it possible to tell at a glance whether amoebae were present or not. Whenever pus was present in the abscess, the amoebae were always found to be accompanied by bacteria. When a large amount of pus was present in the center of the abscess, as was sometimes the case when many bacteria were present, the amoebae were confined mostly to the outer portion near the uninjured tissue. But when no bacteria were present, the entire abscess was hard and dry and the distribution of amoebae was uniform throughout the abscess. In the abscesses which we have studied, there has been no indication that the amoebae ever bring about pus formation. However, if the abscesses were to run a long time—perhaps months or years as they are supposed to do in man—it is possible that pus might be formed.

We have estimated that each of the pieces of the bacteria-free amoebic abscesses which were placed in culture tubes contained from five to ten thousand large active trophozoites. These bacteriologically sterile amoebae have been placed in many kinds of culture media employed in the cultivation of bacteria, all the media that have been used in the cultivation of amoebae, and many others, but in no instance have they lived longer than fourteen days. There was some multiplication in several kinds of media, but the amoebae never multiplied so rapidly as they do when certain bacteria are present. The two kinds of media which gave the greatest promise of successful cultivation were: (1) egg slants covered with horse serum-saline (1–6) with one to three drops of laked blood added to each tube; and (2) liver infusion agar slants covered with horse serum-saline (1–6) with 1 c.c. of hydrolyzed haemoglobin and three drops of the sediment from autoclaved red cells of the horse added to each tube. Many substances were added to each medium; for instance, red cells of the cat, sterile cells from the liver, brain, spleen and kidney of the cat, rice flour, pure rice starch, various kinds of heat-killed bacteria, egg-white, egg yolk, glycogen, glucose, powdered milk, coagulated albumen, etc., but the amoebae were never successfully cultivated. In a few instances the growth of the amoebae appeared to be stimulated somewhat when the oxygen tension was reduced.

We have not spent a great deal of time in cultivating the amoebae with pure cultures of bacteria. In most of the experiments, a medium composed of liver infusion agar slants covered with serum-saline (1–6) and sterile rice flour has been used. After the amoebae had been definitely proved to be free of all bacteria, they were transferred to this medium and then the various bacteria were added. With some bacteria there was little or no multiplication of the amoebae. To this group belong certain of the spore-formers—*Bacillus megatherium, B. cereus* and *B. subtilis*—and *Proteus vulgaris, Escherichia acidi-lactici, Pseudomonas aeruginosa* and *Alcaligines fecalis*. With the spore-formers *B. niger, B. mesentericus* and *B. brevis*, the amoebae grew rather poorly for the first two or three subcultures, but after this they grew better and finally became abundant. They have been grown with *B. brevis* for almost a year and are no doubt capable of growing indefinitely. With *Escherichia communior, Vibrio comma* and *Neisseria catarrhalis* they grow well from the start.

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THE SPECIFIC ACTION OF A BACTERIAL ENZYME ON PNEUMOCOCCI OF TYPE III

A SYSTEMATIC search for enzymes capable of hydrolyzing the polysaccharides found in the capsular material of pneumococci of the various types has been carried on in this laboratory for several years. A number of enzymes from animal and plant sources, known to be active in the hydrolysis of simpler carbohydrates, were tested, but none of them were found capable of attacking the polysaccharides of pneumococcus origin. In addition, cultures of various moulds, yeasts, soil actinomycetes and bacteria, many of which were known to decompose cellulose, were tested without success. Recently, however, a bacillus has been isolated from the organic matter of soil taken from the cranberry bogs of New Jersey, which is able to split the specific capsular polysaccharide of pneumococci of Type III. The micro-organism is

a pleomorphic, Gram negative bacillus, motile and spore-bearing. A detailed description of the special technique employed in its isolation and cultivation, together with a more complete account of its biological characters, will be given in a subsequent publication.

From cultures of this bacillus it has been possible to extract a soluble principle which, in the absence of the living cell, decomposes this specific carbohydrate. The decomposition of the specific polysaccharide is indicated by the appearance of reducing sugars in the hydrolyzed mixtures and by the simultaneous disappearance of serological specificity. The rate of reaction and the total amount of specific substrate decomposed appear to bear a quantitative relationship to the concentration of the active principle.

The active substance present in the sterile bacterial extracts is heat labile, being destroyed by exposure to a temperature of 60° to 65° C. It is extraordinarily specific in its action against the polysaccharide of pneumococci of Type III, since the capsular carbohydrates of the specific types of Friedländer's bacillus, and even those of pneumococci of Types I and II, are unaffected. The fact that the reacting substance is a product of living cells, that it is specific and heat labile and that its action seems to conform to the laws of enzymatic reactions strongly supports the view that the active principle is of the nature of a specific enzyme.

The addition of an active extract to media does not inhibit growth or cause lysis of pneumococci; however, organisms of Type III, when grown under these conditions, are not specifically agglutinable in immune serum of the homologous type. That the *function* of elaborating the type-specific substance is not destroyed, however, is shown by the fact that pneumococci so treated continue to produce the capsular polysaccharide when transferred to a medium devoid of the active hydrolyzing agent. These two facts, namely, the decomposition of the specific carbohydrate removed from the pneumococcus cells and the hydrolysis of the specific capsular substance as rapidly as it is formed in growing cultures are evidence that the active principle is directed against this single, specific component rather than against the cell as a whole.

Previous studies on infection with pneumococci have led to the view that the invasiveness of these organisms is conditioned, in part at least, by the presence of the cell capsule. Since, early in the present work, the experimental evidence pointed to the fact that only the capsular material of the cell is vulnerable to the attack of this enzyme, it was tempting to determine whether the course of pneumococcus infection in a susceptible animal might not be favorably influenced by the injection into the animal of this specific enzyme. This possibility seemed more likely, since it was found that the activity of the enzyme *in vitro* is not inhibited or retarded by the presence of fresh animal serum. Repeated experiments with various preparations of sterile extracts containing the specific enzyme have demonstrated that the active principle has a distinct and specific protective action in mice experimentally infected with pneumococci of Type III. The protection afforded is type-specific, being effective only against pneumococci of this particular type. The protective value of the enzyme is destroyed by heating the bacterial extracts at 70° C. for 10 minutes. The capacity of any given preparation to protect animals against infection bears a definite relationship to its power to decompose the specific polysaccharide *in vitro*.

In addition to its protective action, the active principle has been found to exert a specific prophylactic and curative effect on experimental Type III pneumococcus infection in mice.

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BOOKS RECEIVED

APPLETON, A. B. *Laboratory Guide to Vertebrate Dissection for Students of Anatomy.* Pp. xix + 152. Cambridge University Press. 6s.

CORNER, GEORGE W. *Clio Medica: Anatomy.* Pp. xvii + 82. Hoeber. $1.50.

EVANS, GRIFFITH C. *Mathematical Introduction to Economics.* Pp. xi + 177. 30 figures. McGraw-Hill. $3.00.

FORT, TOMLINSON. *Infinite Series.* Pp. iv + 253. Oxford University Press.

FRANKLIN, WILLIAM S., and G. E. GRANTHAM. *General Physics.* Pp. xvi + 705. Illustrated. Franklin and Charles. $4.00.

HEISENBERG, WERNER. *The Physical Principles of the Quantum Theory.* Pp. xii + 186. Illustrated. University of Chicago Press. $2.00.

HRDLIČKA, ALEŠ. *The Skeletal Remains of Early Man.* Pp. x + 379. Smithsonian Institution.

JULL, MORLEY A. *Poultry Husbandry.* Pp. ix + 639. 229 figures. McGraw-Hill. $4.00.

KAUFFMANN, HUGO. *Allgemeine und Physikalische Chemie.* Pp. 148. 4 figures. Walter de Gruyter & Company, Berlin. RM. 1.80.

THOMAS, CHRISTO. *Cosmogony: A Theory of Cosmo-Dynamics.* Pp. 152. Christopher Publishing House. $1.75.

WARMING, EUGENE, and P. GRAEBNER. *Lehrbuch der ökologischen Pflanzengeographie.* Pp. 240. 99 figures. Verlag von Gebrüder Borntraeger, Berlin. 24M.

WRIGHT, WILMER C., Translator. *Hieronymi Fracastorii de Contagione et contagiosis Morbis et eorum Curatione, Libri III.* History of Medicine Series, No. II. Pp. lvii + 356. Putnam's. $4.50.