ing the washings consumes about two and one half days. This not only removes the microorganisms adhering to the cuticle of the larvae but gives time for those within the larval intestine to be excreted and eliminated. The final washing is always made in Ringer's solution, after which the nematodes are transferred to a special medium. Being unsheathed infective forms, they are now in the stage in which they would normally continue development in the abomasum of the sheep.

The medium consisted of a 0.5 per cent. agar in Ringer's solution containing sheep liver extract, heat-killed ground yeast, sheep blood and sheep kidney, the last either in small pieces or as an extract. The reaction was adjusted to pH 3.0. Two or three drops of defibrinated sheep blood were added to the surface of the semi-solid medium prior to inoculation with several drops of the nematodes suspended in Ringer's solution. The cotton-plugged tubes after inoculation were sealed with sealing wax and incubated at a temperature of 39.5° C.

In numerous tubes examined at intervals during three weeks of incubation, definite development into the parasitic phase has been demonstrable. The presence of new sheaths enclosing the larvae, which were introduced unsheathed into the culture, is an early sign. There is little or no growth during this period, which ends with the ecdysis of the third larval (first parasitic) stage, shown by the presence of cast sheaths and live fourth larval (second parasitic) stages. With subsequent growth and differentiation, these fourth stage larvae have commonly reached a size of 1–2 mm; and some exceeding 3 mm in length have been found. The larger specimens show a well-defined provisional buccal capsule; esophagus 0.3–0.4 mm long; markedly elongated genital primordium, exceeding 200 μ, whose termini can not be easily determined; and clear-cut differentiation into males and females by characteristic configuration of the posterior ends. By comparison with the figures and descriptions of Veglia,[1] these larger forms are advancing into the last third of the fourth larval stage, and by his chronology are comparable to the sixth day of parasitic life in the abomasum. They are about five times the length of the unsheathed larvae with which the culture tubes were inoculated.

We have not seen adult worms in our cultures, but feel confident that these will be obtained with some further slight modifications of the nutritional environment. From the work to date, we do not believe, as has been occasionally concluded by other investigators, that a serious obstacle exists in culturing parasitic stages of the helminths of mammals. The successful *in vitro* culture of the parasitic worms should lead to a more adequate understanding of their physiology and to further elucidation of the mechanism of immunity developed against helminths by their hosts.

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[1] Frank Veglia, 3rd and 4th Reports Dir. Vet. Res. Onderstepoort, pp. 349–500, 1916.

EVIDENCE OF A ROTATIONAL GROWTH FACTOR IN BACILLUS MYCOIDES

IN recent years the question of spirality in animal and plant cells has received wide-spread attention. Little progress has been realized in obtaining an explanation of the phenomenon, but in general it would seem that spirality may arise from the cell itself and is due to the resolution of two growth factors, one longitudinal and one rotational. According to Smith[1] spontaneous growth movements have been said to be due to a wide variety of conditions such as osmotic currents, the action of cilia, peristaltic contractions, protoplasmic streaming and the secretion of gelatinous materials.

Rotation of cells on their long axis is not unknown among the *Thallophyta*. Smith mentions that many of the *Oscillatoria* exhibit such rotations, and Pringsheim and Langer[2] call attention to the well-known twisting in certain of the *Begiotta*.

Hastings[3] has suggested that the characteristic spirality of colonies of *Bacillus mycoids* may be comparable to the spirality in other single-celled organisms, in higher plants and animals. The efforts of numerous bacteriologists to explain the cause of colonial spirality constantly exhibited by *B. mycoides* have failed to produce a convincing explanation. Recently, while studying growing cultures, it was possible to confirm the observations of Pringsheim and Langer concerning the occurrence of spirally twisted filaments of *B. mycoides* (see figure). Evidence obtained from studies on such spiral twists indicates that *B. mycoides* possesses a rotational growth factor which may be responsible for the typical colonial spirality on solid culture media.

A possible explanation for the formation of the rarely occurring spiral twists was sought. Two explanations seemed possible: Either (1) in its normal forward growth the filament of cells moved through the proper planes to eventually form the spiral figure, or (2) the twisted spirals resulted from tension produced by rotation of the cells on their long axes when at some point the filament was so firmly attached that the only relief was to twist or break.

Two methods of establishing conditions under which spiral twists form in great numbers have been successful. In both methods the conditions produced were

[1] G. M. Smith, "The Fresh Water Algae of the United States," McGraw-Hill Book Co., New York, 1933.

[2] E. T. Pringsheim and J. Langer, *Centbl. Bakt.* (etc.), II Ab., 61: 225, 1924.

[3] E. G. Hastings, SCIENCE, 75: 16, 1932.

such that one end of the chain of cells was firmly attached, while the other end was free to move about in a liquid menstruum. The spiral twisting was so rapid that it could be directly observed. It seems that the success in producing twisting filaments under these conditions strengthens the assumption that they form when under stress produced by rotational growth.

Spores of *B. mycoides* were heavily seeded into tubes of liquid 2 per cent. nutrient agar, and thin smears were made from the seeded agar on sterile glass slides resting within petri plates. After solidification of the film of agar, nutrient broth was poured into the petri plate to a depth just sufficient to completely cover the slide and smear. After 24 hours' incubation, the spores had germinated and the tendrils of cells had grown out into the nutrient broth. Spirally twisted loops were present in great numbers. This method of producing subjects for study has the disadvantage of rendering impossible examination with the oil immersion objective, since the slides must be examined while submerged.

A second procedure gave specimens more suitable for close examination. Plates of 2 per cent. nutrient agar were spot inoculated and a colony of two to three centimeters in diameter was allowed to develop. A small rectangular section of the agar, taken near the edge of the growing colony, was transferred to a glass

FIG. 1

slide and a cover slip was pressed tightly down upon the growing cells. The cover glass was sealed to the slide by generous amounts of vaseline, and nutrient broth was injected under the cover slip into the space between the agar block and the vaseline seal. After one to two hours the chains of cells had grown out into the broth and at the intersection of the agar with the broth, spiral twists could be observed forming. The subsequent observations herein reported were made on subjects prepared in this manner.

It was not possible to observe rotational growth by direct microscopic examination of the filament of growing cells. Neither has it been possible to facilitate direct observations of rotational growth by the attachment of particles of silica, charcoal or kaolin to the bacterial cells. It has been possible to determine the direction of rotation of the loop within spirally twisting filaments. Loop rotation is a direct function of the filament rotation. Account was taken of the inverted image given by the compound microscope.

Hundreds of loops of both left (counter clock-wise) and right (clock-wise) spiral strains of *B. mycoides* have been studied and, without exception, loops of left spiral strains rotate from left to right (loop away from the observer, ends of the filament toward the observer), and of right spiral strains from right to left.

The production of new cells in filaments of *B. mycoides* occurs most profusely at the end of the filament. Hence, it is probable that any rotational growth would be most pronounced in the young developing cells which are farthest removed from the attached end of the filament. If this is true, a loop rotation (loop away from observer, ends of the filament toward observer) from left to right would indicate a filament rotation from right to left, when the growing tip is away from the observer and the attached end of the filament is toward the observer. Likewise, a loop rotation from right to left would indicate a filament rotation from left to right under the same condition.

Since left spiral strains invariably give loop rotation (loop away from observer) from left to right, it seems probable that the filament rotation (growing tip away from observer) from right to left is responsible for the left-hand (counter clock-wise) spirals produced by these strains on solid culture media. With the right spiral strains, a filament rotation from left to right is probably responsible for the production of right spiral colonies.

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CARCINOGENICS AND GROWTH STIMULATION[1]

ALL stimulants to growth do not result in cancer, but it seems apparent, as Loeb *et al.*[2] have said, "all causes of cancer directly or indirectly stimulate growth." Goldstein[3] has suggested acceleration of bacterial reproduction as a microbiological test for carcinogenic hydrocarbons. Hammett and Reimann[4] have shown that methyl cholanthrene, a carcinogenic, enhances the production of new growth from anlagen in *Obelia geniculata*. The same authors[5] found proliferation activity of Obelia to be stimulated by the carcinogenic 1:2:5:6 dibenzanthracene.

Our studies, employing planaria (*Euplanaria dorotocephala*) show 1:2:5:6 dibenzanthracene to stimulate both regeneration of cut segments and reproduction of whole animals. In a test period of over a

[1] Published with the permission of the Medical Director of the Veterans' Administration who assumes no responsibility for the opinions expressed or the conclusions drawn by the authors.

[2] L. Loeb, E. L. Burns, V. Suntzeff and M. Moskop, *Am. Jour. Cancer*, 30: 47–53, 1937.

[3] S. Goldstein, SCIENCE, 86: 176–177, 1937.

[4] F. S. Hammett and S. P. Reimann, *Am. Jour. Cancer*, 25: 807–808, 1935.

[5] S. P. Reimann and F. S. Hammett, *Am. Jour. Cancer*, 23: 343–349, 1935.