11247

The Bacteria-Free Culture of a Nematode Parasite.

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It has been possible for some time to grow the entire life cycle of *Neoaplectana glaseri*,[1] a nematode parasite of the Japanese beetle, in cultures in which bacterial and fungous growths have been inhibited in various ways but not eliminated.[2] These contaminants undoubtedly introduced a high degree of variability into the results obtained. It therefore seemed advisable to attempt to rear this parasite in cultures free from bacteria.

Lapage[3] and Glaser and Stoll[4] developed technics whereby the second ecdysis of strongyloid nematode larvae was easily and consistently obtained in quantity under sterile conditions. It was found necessary to modify one of these technics slightly for work with *Neoaplectana*. Cultures prepared in the routine manner were permitted to develop for 10 to 15 days, at which time the majority of the parasites were second-stage larval forms in their third or fourth generation.* These were removed from the surface of the solid medium and washed with water until free of much débris. To remove the dead larvae they were then filtered through 2 layers of lens paper supported by fine gauze. The larvae were ensheathed by aerating them in 25 cc of water for 3 to 4 days with a change of clean water 3 times daily. They were then washed in 25 cc lots of sterile water 3 times each day for 3 days, after which they were treated for 30 minutes to 1 hour with Labarraque's solution (sodium hypochlorite) at a dilution of 1 part to 40 or 50 parts of water. The nemas were again washed 3 times in sterile water, and then placed in

1 Steiner, G., *J. Washington Acad. Sci.*, 1929, **19**, 436.

2 Glaser, R. W., *Science*, 1931, **73**, 614; Circ. 211, Dept. of Agric., State of New Jersey, 1932; *Studies from The Rockefeller Institute for Medical Research*, 1932, **83**, 521; McCoy, E. E., and Glaser, R. W., Circ. 265, Dept. of Agric., State of New Jersey, 1936; McCoy, E. E., and Girth, H. B., Circ. 285, Dept. of Agric., State of New Jersey, 1938.

3 Lapage, G., *J. Helm.*, 1935, **13**, 103; *Parasitology*, 1935, **27**, 186.

4 Glaser, R. W., and Stoll, Norman R., *J. Parasit.*, 1940, **26**, 87.

* The ensheathed second-stage larva is the only stage capable of surviving free in nature and represents the invasive form which must penetrate into a host (Japanese beetle grub) to continue its development.

water about 5 mm deep for from 15 to 20 hours.† The next day the nemas were again treated with Labarraque's solution and then washed 3 times, followed by another 15 to 20 hours' sojourn in a small amount of water. This was followed by a third treatment with Labarraque's solution and 3 more washings with sterile water. Finally, the nemas were again passed through sterile lens paper, to remove any worms that had died during the manipulations, and the viable forms were stored in shallow water until used.

When the above procedures were carefully followed sterile larvae were obtained, shown by the fact that no bacterial growth occurred when they were cultured on standard laboratory media under both aerobic and anaerobic conditions. When occasional contaminants appeared later after a prolonged incubation period, such cultures were either placed aside for collateral observations or discarded.

At first it was thought necessary to have a solid substrate to facilitate the movements and ecdyses of *Neoaplectana.* Neutral veal infusion agar slants may be used, but a simple substrate of 2% agar prepared with 0.5% sodium chloride solution answered just as well. Ten cc of the melted agar were slanted in culture tubes measuring 180 x 22 mm. About one gram of animal tissue, removed under sterile conditions, was then placed at the base of the slant, and 2 or 3 drops of sterile 0.5% salt solution were added. Each tube was inoculated with ± 200 of the previously sterilized second-stage larvae and then sealed by pouring melted sealing wax over the cotton stopper previously trimmed and pushed down into the tube for approximately half an inch. When hard the sealing wax was perforated by a hot wire. Sealing in this manner prevents excessive evaporation without excluding oxygen. Recently we have also sealed many tubes with "Parafilm" perforated with a few needle pricks and have found this satisfactory. The sealed tubes were held in a slanted position and incubated at room temperatures, 22-28°C.

Eighteen- to 20-day-old mouse embryo, beef kidney, and rabbit ovary and kidney have all been found to support growth. The last proved to be the easiest to manipulate and to give the best growth. Consequently it has been used for most of the work. In tubes containing approximately one gram of rabbit kidney the tissue is almost completely digested in from 18 to 24 days; at this same period of incubation, growth has reached its maximum and second-stage larvae predominate. Cultures may be held for at least 3 months without transplantation, but to maintain vigorous growth, transfers are made

† During this time interval *Neoaplectana* digested and evacuated the greater part of its intestinal flora.

every 3 weeks. Sterile nematodes have to date been maintained without loss through 14 transfers or approximately 50 generations. With the methods previously used it was necessary to add certain accessory growth factors in order to maintain the cultures longer than 21 to 32 generations.[5] The nematodes have also been cultured in Erlenmeyer flasks containing 0.5% NaCl solution to a depth of 3 to 4 mm in which are placed pieces of sterile rabbit kidney, and in liquid media containing kidney extracts devoid of particulate matter.

On agar slants containing rabbit kidney the number of nematodes found after incubation for 18 days is approximately 150,000. When grown on Petri dishes, 5.5 cm in diameter, containing 2% dextrose agar and living yeast, the yield was about 40,000. Contamination of the rabbit kidney causes a marked reduction, and in some cases an absence, of growth.

In view of the fact that this parasitic nematode can be grown on both liquid and solid media free from bacteria it will be possible to study its nutritional and other requirements and to test the effects of various vitamins and hormones.

11248 P

Effect of Desoxycorticosterone on Plasma Volume in Intestinal Obstruction.

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Adrenal cortical hormone restores the reduced blood volume in Addison's disease[1, 2] and prevents the drop in plasma volume said to occur during ether anesthesia.[3, 4] This communication reports some experimental observations on the effect of desoxycorticosterone

5 Glaser, R. W., *J. Exp. Zool.*, in press.

1 Thorn, G. W., Howard R. P., and Emerson, K., Jr., *J. Clin. Invest.*, 1939, **18**, 449.

2 Loeb, R. F., Atchley, O. W., Ferrebee, J. W., and Ragen, C., *Trans. Assn. Am. Phys.*, 1939, **54**, 285.

3 McAllister, F., and Thorn, G. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 736.

4 Ragan, C., Ferrebee, J. W., and Fish, G. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 712.