This work was supported by U.S. National Science Foundation grant G–14825.

ROBERT M. EIBEN*
STANLEY M. GARTLER
Department of Medicine,
Divisions of Neurology and Medical Genetics,
University of Washington, Seattle.

* Special trainee, U.S. Public Health Service, BT-594. Present address: Department of Pediatrics, Western Reserve University, Cleveland Metropolitan General Hospital, Cleveland, Ohio.

[1] Krooth, R. S., and Weinberg, A. N., *Exp. Cell Res.*, **25**, 585 (1961).
[2] Krooth, R. S., Howell, R. R., and Hamilton, H. R., *J. Exp. Med.*, **115**, 313 (1962).
[3] Gartler, S. M., Gandini, E., and Ceppellini, P., *Nature*, **193**, 602 (1962).
[4] Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).
[5] Kimura, A., *Exp. Cell Res.*, **23**, 616 (1961).

Prenatal Infection of Bighorn Sheep with Protostrongylid Lungworms

PROTOSTRONGYLID lung nematodes are world-wide in distribution[1], and have been found to influence seriously the health and productivity of domestic animals[2] and may effectively control populations of wild animals[3,4]. Although it is felt that protostrongylids require land snails as intermediate hosts[5,6], all aspects of infection of the final host by these lungworms and related species may not be completely understood.

Several studies have suggested that prenatal infection, which has been reported for other helminths[7], may occur also with lungworms. Eveleth and Eveleth[8], working with domestic sheep, found larvæ of the lungworm *Dictyocaulus filaria* in amniotic and allantoic fluids and in fœtal lambs. Both Pillmore[9] and Buechner[3] gave indirect evidence that prenatal infection by lungworms might occur in Rocky Mountain bighorn sheep, *Ovis c. canadensis*.

The observations reported here were made in western Montana (U.S.A.) to determine if prenatal infection by the lung nematodes *Protostrongylus stilesi* and *P. rushi* occurs in bighorn lambs.

Examination of fæces by the Baermann technique[10] showed that first-stage protostrongylid larvæ were shed by wild bighorn lambs as young as 45–47 days of age. Because the prepatent period for *P. stilesi* has been found to be 30–60 days[11], it would seem likely that these lambs were infected either before or shortly after birth. A captive lamb first began sporadic shedding of small amounts of first-stage protostrongylid larvæ in its fæces when it was five months old. This animal had been removed at one week of age from its native habitat and reared when acquisition of intermediate host snails was not possible[12].

In three lamb necropsies, from one to three first stage protostrongylid lungworm larvæ were found in the parenchymal lung tissue of the right diaphragmatic lobes. The first lamb, born and raised in captivity, was examined ten days after birth. Its mother contained a heavy protostrongylid lungworm infection as determined by fæcal analysis. Another lamb was estimated to be 2–3 days old when found dead in the wild, and came from a herd of bighorns which has a low infection[13] of *Protostrongylus stilesi*. The third necropsy was performed on a near-term fœtus taken from a mature bighorn ewe. This ewe was captured from a wild band of bighorn sheep known to be heavily infected with both *Protostrongylus stilesi* and *P. rushi*[13].

In view of the fact that all larvæ in the lambs mentioned here were found in the right lung, it is interesting to note that in adult bighorn sheep the right diaphragmatic lobe is frequently more infected with lungworm than the left[13]. This has also been reported in protostrongylid infections of domestic sheep in the U.S.S.R.[14]

These results support Pillmore's intrauterine infection theory[9]. However, since only first-stage larvæ were found in the foregoing cases of lung necropsy and this stage is supposedly not infective, other questions are raised concerning the origin and migration of these larvæ and establishment of prenatal infections. Nevertheless, it is significant that immature lungworms were found to enter the definitive host prenatally. To our knowledge this is the first report of prenatal infection by protostrongylid lung nematodes which presumably use land molluscs as intermediate hosts.

This work was supported in part by grants from the U.S. National Science Foundation (G–19422) and the Western Montana Fish and Game Association. We thank the Montana Co-operative Wildlife Research Unit, the Montana Fish and Game Department, Mrs. M. B. Chitwood, Dr. S. N. Boev and Mr. R. E. Pillmore for assistance and advice.

DONALD J. FORRESTER*
CLYDE M. SENGER†
Department of Zoology,
Montana State University,
Missoula.

* Present address: Department of Veterinary Microbiology, University of California, Davis.
† Present address: Department of Biology, Western Washington State College, Bellingham.

[1] Kassai, T., *Acta Vet.*, **7**, 165 (1957).
[2] Boev, S. N., *Lung Nematodes of Hoofed Animals in Kazakhstan* (Acad. Sci. Kazakh. S.S.R., Alma-Ata, 1957).
[3] Buechner, H. K., *The Bighorn Sheep in the United States, Its Past, Present, and Future* (Wildlife Monogr., 4, 1960).
[4] Boughton, R. V., *Canad. J. Res.*, **7**, 524 (1932).
[5] Hobmaier, A., and Hobmaier, M., *Proc. Soc. Exp. Biol. and Med.*, **28**, 156 (1930).
[6] Kassai, T., *Z. Parasitenkunde*, **18**, 5 (1957).
[7] Mozgovoi, A. A., and Nosik, A. F., *Trudy Gel'mint. Lab., Akad. Nauk SSSR*, **10**, 143 (1960).
[8] Eveleth, D. F., and Eveleth, M. W., *Mich. State Coll. Vet.*, **4**, 22 (1943).
[9] Pillmore, R. E., *J. Colo.–Wyo. Acad. Sci.*, **4**, 61 (1959).
[10] Baermann, G., *Geneesk. Tijdschr. Nederl.–Indië*, **57**, 131 (1917).
[11] Pillmore, R. E., *J. Colo.–Wyo. Acad. Sci.*, **4**, 44 (1958).
[12] Forrester, D. J., and Hoffmann, R. S., *J. Mammal.*, **44**, 116 (1963).
[13] Forrester, D. J., and Senger, C. M. (unpublished results).
[14] Schulz, R. S., and Boev, S. N., *Trudy Kazakh. Nauchno–Issled. Vet. Inst.*, **3**, 261 (1940).

MICROBIOLOGY

Mutant Frequency in *Acetobacter*

DURING the past nine years we have demonstrated that most named cultures of *Acetobacter* species obtained from culture collections contained a proportion of *Acetobacter* cells which, on plating, gave rise to colony forms different from those yielded by the bulk of the cells in the culture. On isolating both kinds of colony they were found, in many cases, to differ by only one, or at the most two properties, notably those used in Frateur's classification[1].

The possibility that these mutant culture-constituents might be contaminants, and not mutants, is virtually ruled out by the fact that they were always acetobacters closely resembling the parent culture, as already mentioned. Some of the mutants isolated possessed, indeed, novel combinations of Frateur's criteria, thus being virtually 'new' species.

In addition to the foregoing, freshly isolated and carefully purified *Acetobacter* strains from various types of vinegar were also found, after laboratory maintenance by serial transfer, to become mixtures of two or more different 'species'. Some of such new species have been found highly suitable for large-scale vinegar production.

In a recent paper Schell and De Ley[2] have severely criticized the foregoing findings, on the grounds that we reported higher frequency of occurrence of mutants than they were able to detect, this being because: (*a*) the mutants reported by us might have been contaminants; (*b*) that some 'mutant' colonies might have been merely young colonies of the parent form; (*c*) that other mutant colonies were probably merely 'colony variants'; (*d*) that in general our results "do not constitute a proof that mutation actually occurred as one cannot be sure that no contamination or other mishap occurred during the previous history of the strain".