Table 2. Development of leafhopper under aseptic condition

	Normal condition*	Aseptic condition**				
	Control	1st generation	2nd generation	3rd generation	4th generation	5th generation
Mean nymphal periods (days)	23.3	23.6	23.2	23.4	23.3	23.8
Means survival 30 days (%)	74.8	74.9	74.6	75.0	74.3	74.6
Adult emergence (%)	75.4	75.2	75.0	75.1	75.6	75.4

*Mean value of 10 replicates; 10–18 insects per replicate. **Mean value of 5 replicates; 10–15 insects per replicate.

streak plates derived from these tubes produced no growth. Contaminants isolated from eggs that had not been surface-sterilized were Gram-negative rods, Gram-positive cocci and some fungi.

2. The sterilization tests of old diets. Tests were made on 3 types of old diets as follows:

a) Diet which had been fed to insects for more than 2 days. b) The first diet, but subsequently incubated at room temperature for 3 days. c) The first diet that contained moulted skins.

The diets were all streaked on the 3 kinds of agar plates and incubated at 37 °C for 48 h. All the plates remained clear and without growth. This demonstrates that under aseptic conditions, the insects, and even their droppings and exuviae, always keep clean.

One problem encountered was the drowning of the newly hatched nymphs in the condensed water in the egg chamber. The aseptic conditions necessitated a closed system, so the relative humidity was almost 100% and the water often condensed on the edge of the egg chamber. Some of the newly hatched nymphs drowned before they could jump to the diet. But once on the diet membrane, the insects stayed there most of the time, even during the moulting process. In an undisturbed condition, the insects grew stronger and high humidity was no longer a problem. Actually, high humidity is very favorable for survival. Mitsuhashi[6] stated that for survival of very small nymphs, above 80% relative humidity; the higher the humidity the better the survival.

From results described as above, we found no indication that the normal leafhoppers have gut microorganisms. Also this is supported by Dubroscky[7] based on her histological observations.

1 Present address: Institute of Zoology, Academia Sinica, Taipei, Taiwan 115.

2 T.D.C. Grace, Trans. N.Y. Acad. Sci. (Ser.II) *21*, 237 (1959).
3 K. Maramorosch, Proc. XI. Int. Cong. Entomol. Vienna *2*, 801 (1962).
4 R.F. Hou and M.A. Brooks, J. Insect Physiol. *21*, 1481 (1975).
5 R.F. Hou and M.A. Brooks, Appl. Ent. Zool. *12*, 248 (1977).
6 J. Mitsuhashi, Rev. Pl. Protec. Res. *7*, 57 (1974).
7 I.D. Dubroscky, Contr. Boyce Thompson Inst. Pl. Res. *3*, 39 (1931).

Slime from thermotactic plasmodium of *Physarum polycephalum*

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Summary. Thermotactic plasmodium of *Physarum polycephalum* leaves behind its external layer of slime as it migrates. This rapid tactic movement provides an easy means to collect slime for mechanistic studies.

The plasmodium of *P. polycephalum,* an acellular slime mold, is an attractive system for phenomenological and behavioral studies of nonmuscle motility. The organism migrates by a net flow of cytoplasm through incessant shuttle streaming[1]. With speed reaching 1350 microns/sec at its maximum, the cytoplasmic flow of this organism has been recognized to be the highest in all motile systems[2]. The direction of migration can be random or dictated by external stimuli including light, heat, electricity and chemicals[3–8]. Although much information exists indicating actin and myosin as major components of the machinery of the shuttle streaming, very little is known about how the mechanism may be controlled[9–11]. In the cellular slime mold, *Dictyostelium discoideum,* however, it has been suggested that a gradient in the thickness of the surface sheath plays a role in the control of directional motion as well as in morphologensis[12]. Similar suggestions that slime may indeed possess certain physiological activities instead of a merely inert secretion have been reported in *Physarum* as well as *Dictyostelium* species[13–16]. In addition to slime, Anderson observed that a migrating plasmodium has a potassium gradient[17]. His assays indicated that a higher potassium content was found in the anterior region of the plasmodium. There was no attempt reported to identify the exact location of this ion, hence leaving a possibility that the potassium ion may actually be left in the slime fraction of the anterior region of the migrating plasmodium. Even though the chemical components of the slime have been preliminarily investigated by Simon and Henney, Jr[18], their samples were obtained from microplasmodia grown in liquid culture with proper agitation, and were separated from other cellular components by ethanol precipitation. There is no evidence that the microplasmodia grown in such an environment were exhibiting oriented migration. Whether slime is involved, or some other component (such as potassium ion) is involved in controlling directional movement similar to that found in *Dictyostelium,* requires a chemical study of slime collected from a 'migrating' plasmodium. We report here such a simple separation method.

Essentially the plasmodium was handled as reported in a previous publication[4]. The plasmodium of *P. polycephalum* M_3C was grown by spreading a suspension of microplasmodia onto a 25 mm millipore filter laid on the surface of agar supplemented with semi-defined growth medium with hemin and citrate[19]. The filters, when covered with freshly grown plasmodia at log phase, were lifted and excess

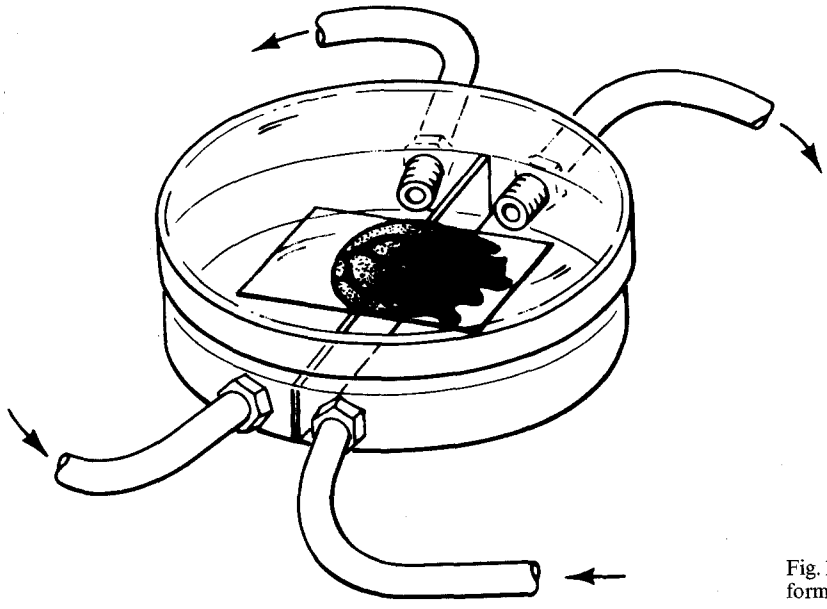

Fig. 1. A schematic drawing of a plasmodium performing thermotactic migration in the apparatus.

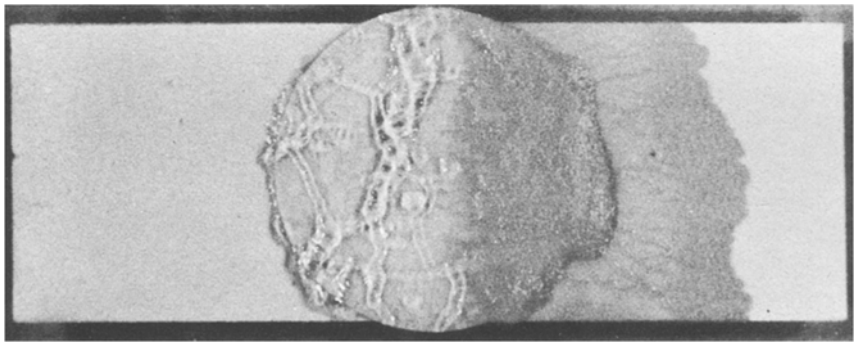

Fig. 2. Plasmodium responded to a 10 °C temperature gradient (20 °C on the left and 30 °C on the right) by spreading its cytoplasm towards the right (favorable condition) and leaving behind shiny slime on the left half of the membrane support. The response was detected at the end of a 30-min thermotactic drive.

nutrients were washed off by floating the filters on distilled water and drying them on blotting paper 3 times. Each individual organism supported by membrane filter was then subjected to a thermotactic treatment.

A simple apparatus designed for thermotaxis study was constructed by cementing a plastic Petri dish on top of another one having a midline partition, thus 2 chambers were established in which the temperatures can be maintained by controlling the temperatures of the water circulating in each of the 2 chambers (figure 1). In the observation dish, a double filter strip (Millipore on top of Whatman, cut to equal size) was placed across the midline temperature zone and wetted with water to provide moist support for the migrating plasmodium. The organism in its membrane support was laid in the centre of the double filter strip with its plasmodium stretching across the temperature gradient. In approximately half-an-hour, the plasmodium was driven to a favorable temperature in the gradient, leaving behind an optically clear layer of slime on the membrane filter (figure 2). After the plasmodium had vacated the unfavorable temperature region, the membrane support was pulled further toward the unfavorable temperature region to re-establish the original temperature gradient necessary for driving the organism ahead. Eventually, the whole organism moved away form its original support leaving behind a layer of slime. This membrane support was removed for chemical investigation.

The separation method described gives slime from a 'migrating' plasmodium with known direction of migration. No chemical precipitation is employed for separation, and the slime sample thus obtained is especially suitable for a study of the distribution of chemicals along the migration direction. In the event, an analysis of chemicals distributed in the migrating direction is desired, the slime can be scraped off in sections and analyzed accordingly.

1 W. Seifriz, Bot. Rev. *9*, 49 (1943).
2 N. Kamiya, Cytologia *15*, 183 (1950).
3 M. Hato, T. Ueda, K. Kurihara and Y. Kobatake, Cell Struct. Function *1*, 269 (1976).
4 W.-W. Tso and T.E. Mansour, Behav. Biol. *14*, 499 (1975).
5 J.D. Anderson, J. gen. Physiol. *35*, 1 (1951).
6 I. Tasaki and N. Kamiya, Protoplasma *39*, 333 (1950).
7 M.J. Carlile, J. gen. Microbiol. *63*, 221 (1970).
8 D.R. Coman, Arch. Path. *29*, 220 (1940).
9 S. Hatano and F. Oosawa, Ann. Rep. Res. Group Biophys. Japan *II*, 29 (1962).
10 S. Hatano, Adv. Biophys. *5*, 143 (1973).
11 K.E. Wohlfarth-Bottermann and M. Fleischer, Cell Tissue Res. *165*, 327 (1975).
12 W.F. Loomis, Jr, Nature New Biol. *240*, 6 (1972).
13 L. Cheung, H.R. Henney, Jr, and W.H. Clark, Jr, Cytobios *9*, 193 (1974).
14 H.R. Henney, Jr, and M. Asgari, Can. J. Microbiol. *21*, 1866 (1975).
15 H. Yamada, I. Suzuki, Y. Kumazawa, Y. Kawamura, K. Mizunoe, Y. Aramaki and T. Miyazaki, Biochim. biophys. Acta *538*, 627 (1978).
16 H. Yamada, Y. Aramaki and T. Miyazaki, Biochim. biophys. Acta *497*, 396 (1977).
17 J.D. Anderson, J. gen. Physiol. *45*, 567 (1952).
18 H.L. Simon and H.R. Henney, Jr, FEBS Lett. *1*, 80 (1970).
19 J.W. Daniel and W.W. Baldwin, Meth. Cell Physiol. *1*, 9 (1964).