

vival is highly significant ($P < 0.01$) when compared to development of the uninjected control eggs. Probably the decreased survival resulted from the increased volume of injected fluid or the increased quantity of injected protein. In some, though not all, microinjected amphibian eggs, a relation has been shown between developmental arrest and the amount of injected cytoplasmic globulin from amphibian liver (15).

The fetuses which developed from the microinjected eggs were smaller more frequently than were their native littermates (Fig. 4), although no other external abnormality was observed. Among 23 living fetuses developed from injected eggs, 6 smaller fetuses were found, whereas, in the control groups, only 1 out of 19 donor fetuses was smaller in size. From the present data, one cannot definitely conclude that microinjection affected fetal size; however, the difference of frequency was not far from a statistically significant level ($P = 0.0801$; Fisher's Exact Test).

The number of degenerating embryos and resorption sites was also examined in the autopsied recipients. Fifty-seven nonpunctured, uninjected, fertilized eggs were transferred into the left oviduct of 13 pregnant recipients. The difference in the number of degenerated embryos in each uterine horn was not statistically significant. However, out of 244 eggs punctured by the injecting pipette and transferred into the left oviducts of 55 pregnant mice, 53 degenerated embryos were detected in the left horns but only 34 were present in the right horns. The degenerated embryos detected in the right uterine horns were derived entirely from eggs of the recipients while those in the left horns were probably derived from both native and transferred eggs. Thus, the higher degeneration which occurred in the left uterine horns of animals receiving injected eggs may be due partly to the degeneration of some transferred injected eggs after implantation (P close to 0.05).

Amphibian eggs, because of their large size, have long been the favorite material for various microaspiration and injection experiments (15, 16); often, however, they have been obtained from animals of undefined genetic background. Although mammalian eggs are comparatively much smaller, these data demonstrate that injection of the mouse egg at the

pronuclear stage can be successful and that a fair number of eggs survive micromanipulation and injection of a foreign substance. A useful method is thus provided for observing the developmental or hereditary effects of injected materials on ova from genetically defined mice or other mammals.

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Light Sense in Nematodes

Abstract. *The stimulating effect of daylight on the oxygen uptake of the infective larvae of Haemonchus contortus and Nippostrongylus brasiliensis, measured at constant temperature, provides the first proof of a dermal light sense in the Nematoda. Nippostrongylus larvae are less sensitive than those of Haemonchus and require very high light intensities before a significant effect can be detected.*

It has not been proved so far that the Nematoda have a dermal light sense (1), though some behavioral studies (2, 3) have suggested that phototaxis or photokinesis are involved in the natural accumulations of certain roundworm infective larvae. Proof of a light sense of this kind, however, is unlikely to emerge from behavioral or population studies on their own, since the design of such experiments can rarely eliminate the effects of other variables (4, 5).

Clear evidence of a different kind is reported here for the presence of photosensitivity in the free-living third-stage larvae of a ruminant parasite, *Haemonchus contortus*. I find that the oxygen uptake of these larvae is significantly altered by change in light intensity above a threshold value. Light-adapted larvae also consume more oxygen than dark-adapted ones (Fig. 1A; Table 1). Infective larvae of the rat parasite, *Nippostrongylus brasiliensis*, placed in the same situation show little evidence of light sensitivity (Fig. 1B) unless the illumination is very bright, when small but significant effects are seen (Table 1). In normal laboratory daylight illumina-

tion, no significant difference in oxygen uptake between light-adapted and dark-adapted *Nippostrongylus* larvae can be detected. Inasmuch as no structures which could be construed as special light receptors have been described in these species, the sensitivity demonstrated may be classed as a "dermal" one (1).

My earlier experiments (6) show that the oxygen uptake of *Nippostrongylus* larvae is profoundly influenced by a change in temperature, a period of 2 to 3 hours being necessary before the response to a rise in temperature subsides. A similar response to temperature change appears to exist in *Haemonchus* larvae and initially overrides differences due to light intensity when larvae are raised from ambient temperature to that of the Warburg bath (Fig. 1A, first 100 minutes). *Haemonchus* larvae kept in light intensities below 200 lu/m² (that is, well below the threshold) for 18 hours and subsequently raised to 30°C without change in light intensity show a similar decline in oxygen uptake. The threshold intensity of diffuse daylight for a response in *Haemonchus* is estimated to be between 530 and 1200 lu/m². Differ-

ent populations of *Haemonchus* larvae show significant variations in the magnitude of the response (Table 1). The reasons for this are as yet unknown.

Larvae for these experiments were cultured and isolated by the method of Christie and Patterson (7) for *Hae-*

Table 1. Oxygen uptake at 30°C, per milliliter of larval suspension per hour, under different light conditions. Experimental design is same as described in legend of Fig. 1. Hc, *Haemonchus contortus*; Nb, *Nippostrongylus brasiliensis*.

Approx. light intensity (lu/m ²)	Oxygen uptake (μl)			
	Exptl. flasks		Control flasks*	
	1 hr before†	1 hr after†	1 hr before†	1 hr after†
<i>Hc</i> (experiment 1)				
3200	10.1	34.4	19.2	16.2
	10.5	39.6	18.5	17.3
<i>Hc</i> (experiment 2)				
7000	21.7	47.7	30.9	30.2
	21.9	46.3	29.5	33.1
<i>Nb</i> (experiment 1)				
3200	44.6	46.5	43.2	43.8
	44.8	45.7	39.9	41.4
	42.4	47.9	42.5	42.5
<i>Nb</i> (experiment 2)				
6700	26.9	32.4	29.4	26.8
	25.7	28.8	29.6	27.3
	27.3	29.1	31.4	30.1

* Control flasks were in light throughout the experiments. † Before and after aluminum foil was removed from experimental flasks.

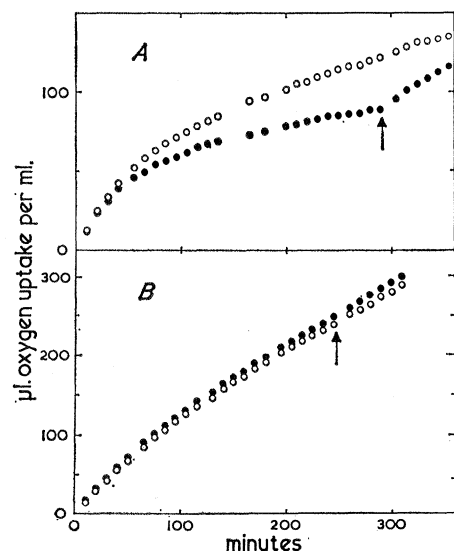


Fig. 1. Oxygen uptake at 30°C of *Haemonchus contortus* (A) and *Nippostrongylus brasiliensis* (B) infective larvae in daylight of approximately 3200 lu/m² (○) and in the dark (●). The arrows indicate where larvae in darkness were subjected to light by removal of the protecting aluminum foil. Each point is the mean of two (A) or three (B) 6-ml Warburg flasks containing 1 ml of larval suspension in simple antibiotic solution (A) and 1/4 RLA/solution (B).

monchus and that of Wilson and Dick (8) for *Nippostrongylus*. *Haemonchus* were accumulated in water at 5° to 7°C for periods of 7 to 10 days. For a day before respirometry, and during the experiments themselves, they were contained in an antibiotic solution (penicillin and streptomycin, 50 mg/liter each; and actidione, 20 mg/liter), being washed 3 times initially and again immediately before distribution to the Warburg flasks. *Nippostrongylus* were kept on a shaker at 25°C in a balanced salt solution containing the same antibiotics at the above concentration ("1/4 RLA+") (9) or at half strength ("1/8 RLA") (6). Experiments with *Haemonchus* in water alone confirm that the response is confined to the larvae and is not a light-reversible inhibition of oxygen uptake by the antibiotics.

Warburg constant-volume manometric techniques were used to measure oxygen uptake at constant temperature (30°C in all cases; Braun system); and the larvae were subjected to darkness by enclosing the respirometer flasks and manometer arms in aluminum foil. The Warburg bath was constructed of transparent plastic and therefore allowed the maximum amount of light to reach larvae in uncovered flasks. The light source was diffuse daylight in a laboratory with windows facing northeast. Unless artificially diminished, the incident light falling on the apparatus varied between 2120 and 7600 lu/m² during the experiments.

Absence of an effect of light on oxygen consumption does not, of course, rule out all types of photosensitivity. Photokinesis, such as that reported for *Trichonema* larvae (3), need bear no relation to changes in metabolic rate. Thus *Haemonchus* and *Nippostrongylus* larvae may respond in some way to light below the threshold necessary to stimulate oxygen uptake. In the case of *Nippostrongylus*, however, the very high intensities of illumination necessary to bring about even small changes in oxygen uptake in my experiments, together with the conclusions of Parker and Haley (5) from behavioral studies, suggest that light sensitivity is of little consequence in its biology. Nevertheless, the demonstration of such a stimulus in both species under conditions of rigorous temperature control constitutes proof for the first time of a dermal light sense in nematodes.

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Fluctuation Tests with Antibody-Forming Spleen Cell Populations

Abstract. Shortly after immunization of mice, cells forming specific antibodies to one antigen, for example, sheep red blood cells, are nonrandomly distributed throughout the spleen. If the spleen donor has been immunized with two different antigens, for example, sheep red blood cells and chicken red blood cells, the nonrandom distribution of spleen cells forming antibody to one antigen differs significantly from that of cells forming antibody to the other antigen. These findings are in accord with a clonal distribution of antibody-forming cells.

Jerne's plaque technique, which permits an estimation of the number of spleen cells forming specific antibodies (1), has made it possible to apply the fluctuation test, previously used in assays on bacterial populations (2), to populations of antibody-forming mouse cells. By comparison of the fluctuation due to sampling error, obtainable by plating several samples from one spleen piece, with the fluctuation in numbers of antibody-forming cells in different pieces of the same spleen, it was determined whether formation of antibodies to a given antigen was randomly distributed throughout the spleen or tended to occur in clones of antibody-forming plasma cells.

Mice (AKR) were immunized intravenously with 1×10^8 sheep red blood cells, and their spleens were removed 72 hours later. The spleen was cut into 24 pieces of approximately equal size by two longitudinal and seven crosswise cuts. Cell suspensions were prepared from each piece, counted in a hemocytometer, and assayed for number of