rated with a loading dose of its substrate, more of this intermediary kynurenine accumulates. This is the result of enzymic imbalance: the larger amounts of kynurenine produced by more tryptophan pyrrolase exceed the unchanged capacity of enzymes that catabolize it. Another enzymic imbalance, but with a different mechanism, is responsible for the increased excretion of kynurenine that accompanies vitamin-B_6 deficiency; kynurenine is produced in normal amounts but accumulates because of the low activity of pyridoxal phosphate–requiring enzymes (such as kynureninase) that metabolize it.

Increased excretion of tryptophan metabolites, in the amounts found by us after administration of hydrocortisone, have been reported in patients having rheumatoid arthritis, schizophrenia, renal tuberculosis, Hodgkin's disease, scleroderma, and various other diseases (*8*). Many of these diseases may be associated with "stress", which results in increased adrenocortical secretion by stimulation of the pituitary-adrenal axis. There may also be greater sensitivity to the adrenocortical hormones in some of these disease states. Our results demonstrate correlation between the activity level of tryptophan pyrrolase in liver and the amount of kynurenine in urine, and also show that the tryptophan pyrrolase is induced in man by hydrocortisone. It is therefore possible that induction of tryptophan pyrrolase by adrenocortical hormones may represent the common factor leading to increased excretion of tryptophan metabolites in diverse diseases in man.

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Microinjection of Mouse Eggs

Abstract. *A technique has been developed for injecting known amounts of liquids into fertilized one-celled mouse eggs by use of a calibrated ocular micrometer superimposed on the terminal regions of the injection pipette. From 128 pronuclear eggs, each injected with 180 or 770 cubic microns of bovine gamma globulin at a concentration of 25 milligrams per milliliter in citrate–Locke's solution and then transferred to the oviducts of pregnant foster mothers, 18 living fetuses developed; from 74 eggs, each injected with 2730 cubic microns, 5 fetuses survived. The living fetuses that developed from the injected eggs were smaller than normal in 6 of 23 surviving experimental fetuses as contrasted to only 1 of 19 control fetuses.*

Microinjection of living cells other than mammalian eggs has been practiced for about half a century (*1*). Micrugical studies have even utilized the living eggs of the rat (*2*), rabbit (*3*), mouse (*4*), and human (*5*) during the last two decades, but the methods have not been able to safeguard the structural or functional integrity of the whole ovum. The mammalian egg is

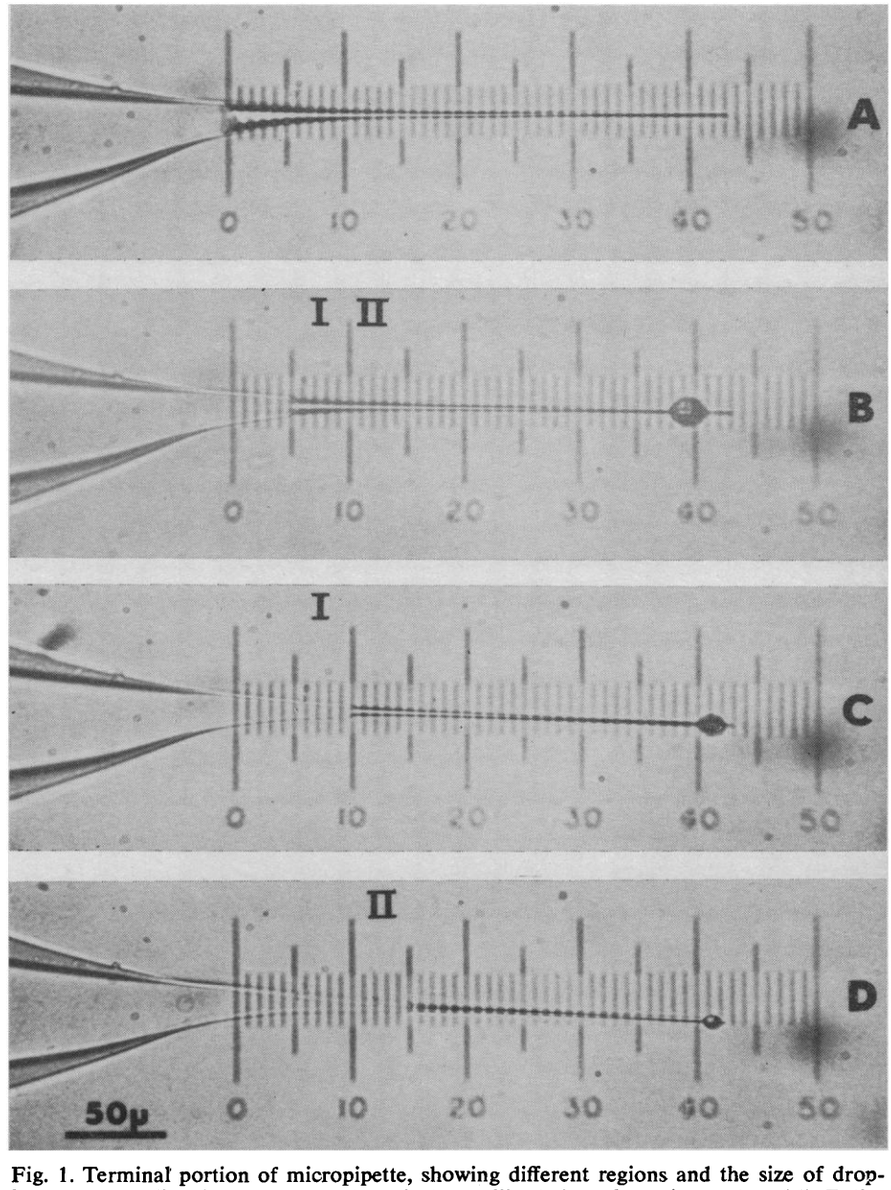

Fig. 1. Terminal portion of micropipette, showing different regions and the size of droplets corresponding in measurement against a calibrated ocular micrometer. (*A*) Entire terminal portion of pipette filled with solution; (*B*) upper region (2730 μ^3); (*C*) middle region I (1340 μ^3); and (*D*) middle region II (180 μ^3). Details in text.

apparently more difficult to inject than other cells because of the thick zona pellucida and the vitelline membrane, both of which are highly elastic and resistant to the penetration of a microinstrument, especially at the unfertilized stage (*6*). This investigation was undertaken to surmount these obstacles and to develop a technique for successful microinjection of the mouse egg.

At the scheduled time of hormonally induced ovulation (*7*), female mice of the black-agouti C3H/HeJ strain were caged with fertile males of an albino strain, either BALB/c or Cal A (originally A/Crgl/2). Nine to twelve hours after the expected mating, eggs were flushed from the oviducts, and fertilized eggs were identified by the presence of the second polar body or by observation of pronuclei.

These fertilized eggs were then placed in a medium consisting of three parts modified Locke's solution, one part 2.9 percent sodium citrate dihydrate, and 25 mg of bovine gamma globulin (BGG, Armour) per milliliter of the citrate–Locke's solution. Penicillin (100 unit/ml) and streptomycin (50 μg/ml) were added to the medium. Viscosity of the medium at 22°C was 1.1591 cp and its *p*H, 7.2. Eggs were retained for micromanipulation and injection within a drop of the bovine gamma globulin–citrate–Locke's solution (GCL) which was covered with mineral oil in a vaseline well on a microscope slide.

One unit of a Leitz micromanipulator held the "egg-holder" pipette (see *8*) which had an outside diameter of 40 to 60 μ and, after fire-polishing, an inner diameter of 15 μ. The opposite unit of the micromanipulator held the injecting pipette, the tip of which was tapered over a terminal length of about 250 μ from a diameter of 25 μ to about 1 μ at the inner orifice (see *9*). The outside diameter of the entire tip was not greater than that of the head of a mouse sperm. The injecting pipettes were stiff and pointed since slender, flexible, blunt micropipettes bend or create an indentation when

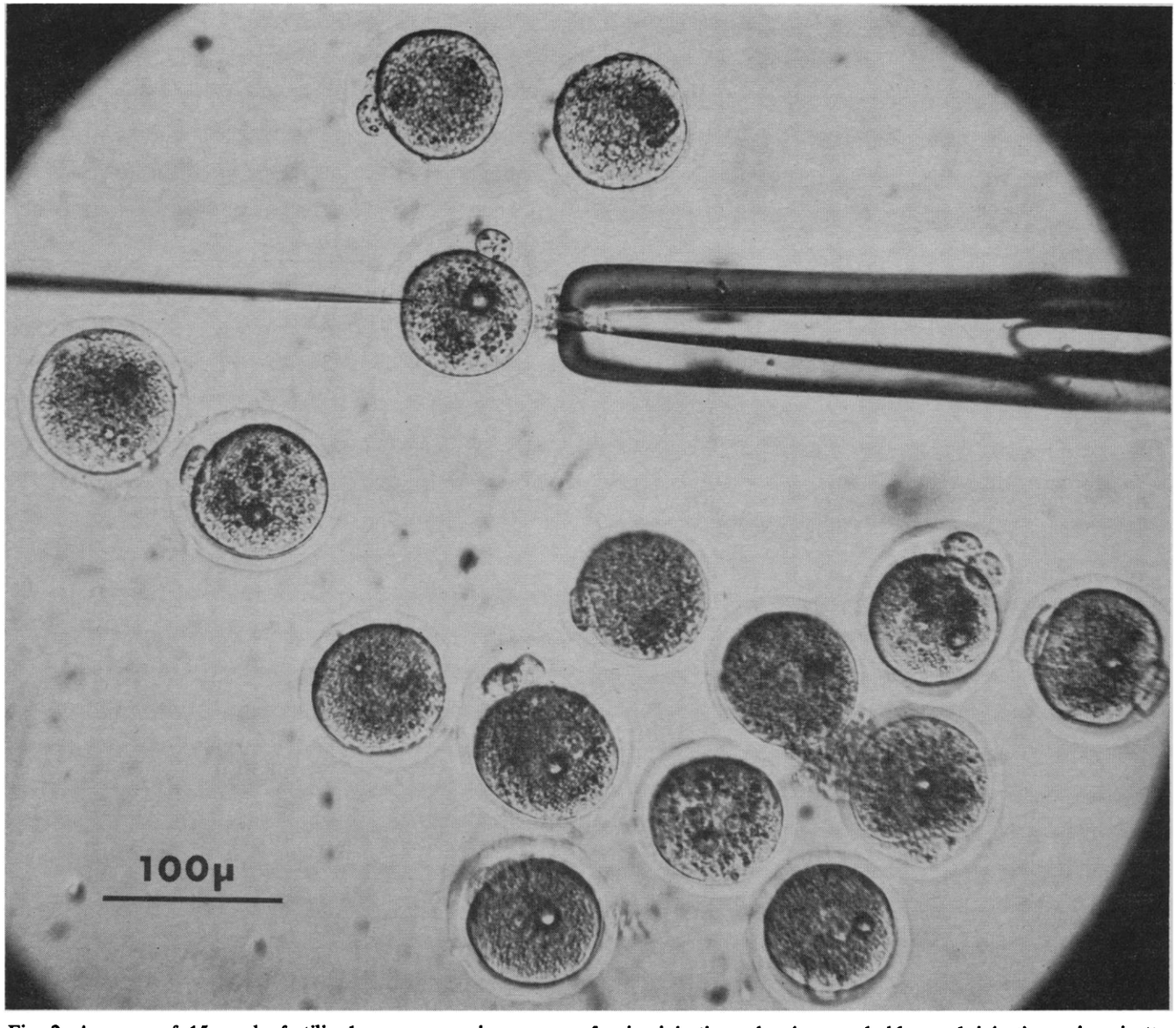

Fig. 2. A group of 15 newly fertilized mouse eggs in process of microinjection, showing egg-holder and injection micropipette. Thirteen eggs have been injected with an oil droplet; above the micropipettes are two uninjected eggs. On the extreme left one egg has been damaged by injection and its vitelline membrane has lost the capacity to shrink.

pressed against the zona pellucida of the mammalian egg.

The egg-holder was connected by polyethylene tubing (Clay-Adams PE 60) to a screw-controlled syringe. The injecting pipette was similarly connected to a screw-syringe held by a Wells microinjector (*10*). The complete microinjector set thus had two controls: the control in the Wells injector could regulate delivery of a large volume of injection medium and the control in the screw-syringe could regulate delivery of a small volume. The egg-holder and the injecting pipette first were filled with mineral oil to the tip and then both were further filled with GCL test solution by capillary action from the drop of medium holding the eggs in the vaseline well. By this means pipettes could be filled as many times as required for injecting many eggs. The egg was maintained in position during its injection by negative pressure in the holder pipette and was released by increasing the pressure in the micrometer syringe.

The volume of each injection was measured by a calibrated ocular micrometer superimposed on the terminal of the injecting pipette which was divided into four regions: upper, two middle regions, and the tip end; each region was subdivided as shown in Fig. 1. The volume delivered by each division of the pipette was predetermined by measuring the diameter of an aqueous droplet surrounded by mineral oil at the tip of the pipette. The holding capacity of the pipette terminal was determined to be about 5000 μ^3; thus, the amount of BGG in this volume from the known dilution (25 mg/ml) was calculated to be about 125 picograms (1 $\mu^3 = 10^{-12}$ ml; 1 pg = 10^{-9} mg).

Three hundred and one newly fertilized eggs were maintained in vaseline well preparations and each received one of the following experimental treatments: (i) no pipette puncture and no injection; (ii) puncture of egg but no injection; (iii) injection of 180 μ^3 of medium solution containing about 5 pg of BGG; (iv) injection of 770 μ^3 of the solution containing 20 pg of BGG; or (v) injection of 2730 μ^3 of the solution containing 68 pg of BGG.

Viability of eggs was tested by transplantation into mated inbred albino BALB/c or Cal A recipients which were under anesthesia (*11*). The recipients had been induced by hormone injection to ovulate simultaneously with the agouti C3H donors and had been mated with fertile males of their own albino strain. Injected or uninjected experimental eggs were pipetted into the left oviduct of the recipient through a small opening in the membrane of the ovarian bursa; a small air bubble in the pipette was used as a visible marker to indicate when all the transferred eggs had been released into the infundibulum. Each albino recipient received four or five normal-appearing experimental eggs. Recipients were autopsied between the 17th and 19th day of gestation in order to avoid possible destruction of the young after birth by the foster mother. Fetal eye pigment enabled transferred and recipient native fetuses to be distinguished from one another.

The entire procedure from the time experimental eggs were flushed from the donor oviduct to the time of their transplantation into the recipient infundibulum required 30 to 40 minutes. During this period of maintenance in vitro at room temperature many eggs shrank within their zonae pellucidae (Fig. 2). Although shrinkage did not occur during shorter exposures at room

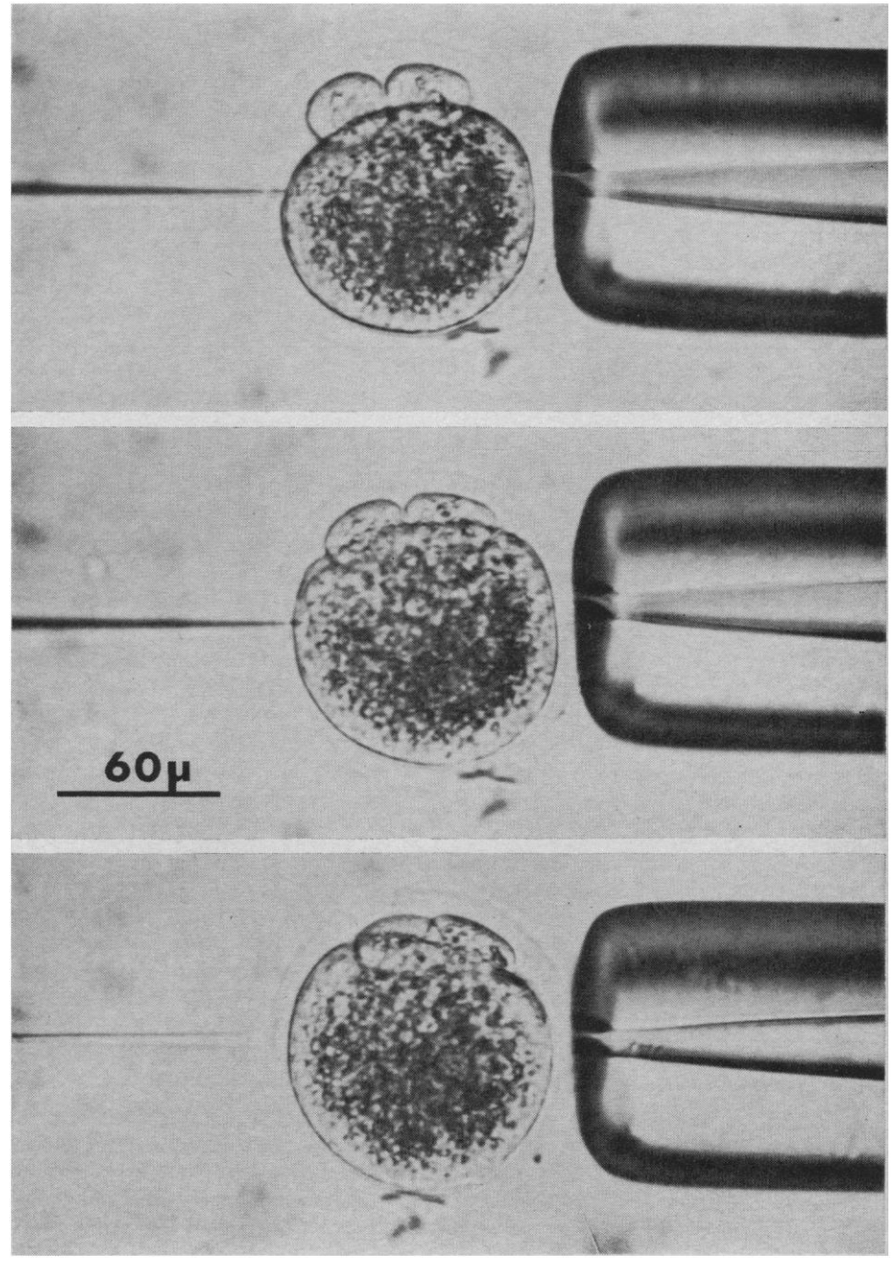

Fig. 3. Microinjection of a fertilized mouse egg with bovine gamma globulin solution. (Top) Holding of egg and pipette penetration. (Middle) Expansion of vitellus by over-injection. (Bottom) Withdrawal of micropipette after injection. Details in text.

temperature, the shrunken eggs usually survived (*12, 13*) and were regarded as essentially normal.

The zona pellucida and vitelline membrane of unfertilized mouse eggs were elastic, and the ooplasm was watery (*6*); in contrast, the coverings of a fertilized pronuclear egg were less elastic and the vitellar substance was more viscous. Microinjection was easier at the pronuclear stage than at the unfertilized stage. During injection, movement of cytoplasmic granules near the tip of the injecting pipette was clearly visible. When a pipette passed through the zona pellucida, but did not get into the vitellus, a deep indentation of the vitelline membrane was formed; in such cases, injected solution flowed into the perivitelline space and an oil droplet was present in the indentation. Oil droplet "coalescence" with the egg as described for sea urchin (*14*) was not observed in mouse ovum. When the pipette did not penetrate the vitelline membrane, the oil droplet was always expelled as the pipette was withdrawn.

Table 1. Development of mouse eggs after microinjection and transplantation by way of left infundibulum. GCL, bovine gamma globulin–citrate–Locke's solution; S.D., standard deviation; NP, no puncture; and P, puncture.

Av. vol. GCL injected per egg (μ^3, ± S.D.)	Pregnant recipients (No.)	Ova transferred (No.)	Living donor fetuses developed		Degenerated embryos (No.)	
			No.	Ova transferred (%)	Left horn	Right horn
0.0 (NP)	13	57	13 (1)*	23	10	11
0.0 (P)	10	42	6 (0)	14	8	5
180 ± 80	15	71	11 (1)	15	14	8
770 ± 150	13	57	7 (3)	12	13	7
2730 ± 570	17	74	5 (2)	7	18	14

* Numbers in parentheses indicate small fetuses.

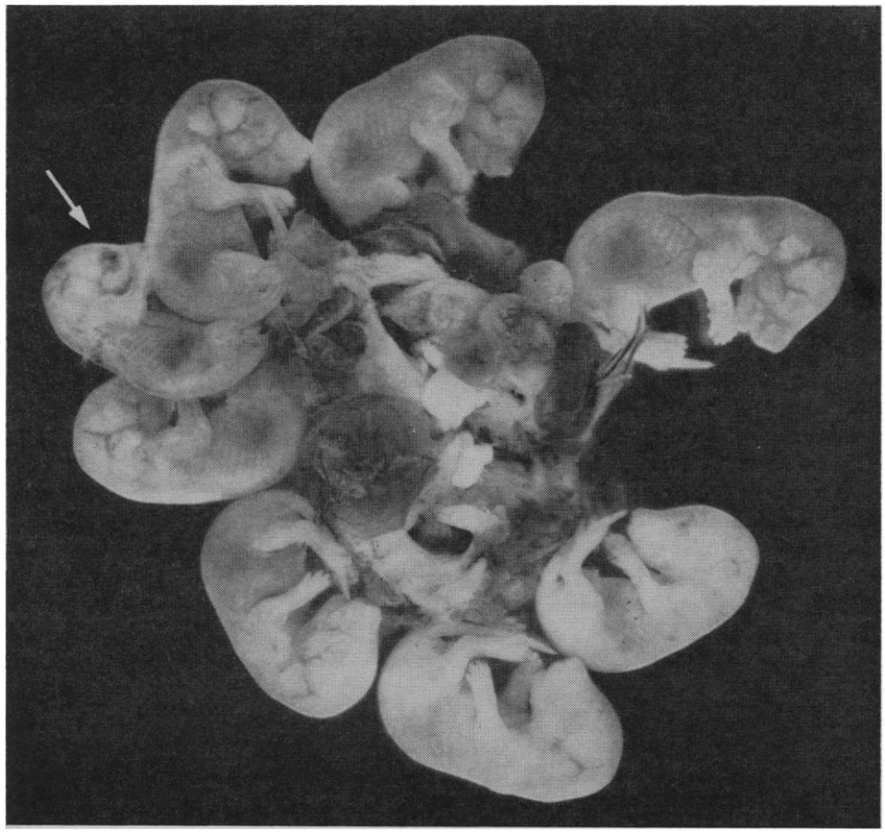

Fig. 4. A litter of 17-day-old fetuses. One dark-eyed fetus (arrow) developed from transplanted eggs injected with 2730 μ^3 of bovine gamma globulin solution prior to transfer. Compare the dark-eyed fetus with its native littermates for size.

Immediately after the pointed pipette pierced through the membrane, both the zona pellucida and the vitelline membrane resumed their normal contour and the egg was restored to its original appearance (Fig. 3, top). However, if an egg was injured by the micropipette, the vitelline membrane lost its ability to shrink and the vitellus rapidly became disorganized.

The volume of a spherical mouse egg 70 μ in diameter was calculated to be about 179,600 μ^3, and injection of fluid of approximately 770 μ^3 did not cause any detectable distortion of the vitellus. Injection of approximately 2730 μ^3 of fluid, however, distended the egg, and the vitellus expanded to fill the perivitelline space, pressing the polar bodies closely against the zona pellucida (Fig. 3, middle and bottom).

The survival of pronuclear eggs after the various treatments is shown in Table 1. Three hundred and one treated eggs of agouti C3H females were transplanted into 68 pregnant albino females. Thirteen dark-eyed living fetuses developed from 57 nonpunctured, uninjected eggs (first control) put into 13 pregnant albino recipients (23 percent survival). Forty-two punctured but uninjected eggs (second control) were transplanted into 10 pregnant albino recipients; from these experimental controls, 6 living fetuses (14 percent) were obtained. Injection of saline into eggs, as another form of control, was discontinued because of contamination of saline with GCL test solution due to capillary refilling action at the tip of the micropipette.

Seventy-one eggs, each injected with 180 μ^3 of GCL, showed no more damage than those eggs which were punctured but uninjected; after transplantation into 15 recipients, 11 (15 percent) of these were normal living fetuses at autopsy. Of another group of 57 eggs, each injected with 770 μ^3, only 7 fetuses (12 percent) survived to near term. Differences in survival rates between these two groups of injected eggs and the second control group were not statistical significant.

When injected with 2730 μ^3, the eggs became slightly distended and survival rate dropped; only 5 living fetuses (7 percent) resulted from 74 such eggs transplanted into 17 pregnant females. This reduction in sur-

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 basiliensis, *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. 1*A*; Table 1). Infective larvae of the rat parasite, *Nippostrongylus brasiliensis,* placed in the same situation show little evidence of light sensitivity (Fig. 1*B*) unless the illumination is very bright, when small but significant effects are seen (Table 1). In normal laboratory daylight illumination, 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. 1*A*, first 100 minutes). *Haemonchus* larvae kept in light intensities below 200 lu/m^2 (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/m^2. Differ-