with constant mixing and changing of the saline. On incubation no mitosis-stimulating activity was found.

In a control study RNA was extracted in the same manner from lymphocytes (from the same donor) which had not been incubated with the various specific antigens. When this was added to fresh autologous lymphocyte cultures no fraction showed mitotic activity.

Further controls consisted of lymphocyte cultures from the same donor; to one set nothing was added, and these cultures gave uniformly negative results. On the other hand, controls to which phytohemagglutinin was added were uniformly positive.

Each culture was scored for cell transformation and mitosis from counts of 1000 cells. The result was considered positive when cell counts indicated more than 3 percent transformation and over 0.1 percent mitosis (Fig. 2). Only the lymphocytes, which had been challenged with specific antigens to which the donor was presumably sensitized, were capable of stimulating autologous unchallenged lymphocytes to transform and divide. The same mitosis-stimulating and transforming activity was obtained with the total RNA extracted from the stimulated lymphocytes, the interface RNA, and fractions I and II of the sucrose gradient. The activity thus appeared to reside in the heavy, ribosomal RNA fractions. The fact that the same RNA fractions from unstimulated lymphocytes did not stimulate mitosis suggests the possibility that the active ribosomal fractions may have formed complexes with, or somehow have been altered by, the challenging antigen.

It remains to be demonstrated, however, whether the ribosomal RNA which is responsible for the transforming and mitosis-stimulating activity resides in that portion of the ribosomes which are rapidly labeled as opposed to the heavier ribosomes which are not rapidly labeled in growing cultures.

It is not known yet whether cytoplasmic connections occasionally seen (Fig. 1) between transforming cells and small lymphocytes form a bridge of functional continuity caused by cytochemotaxis to allow for transport of particular material between an immunologically competent small lymphocyte and a newly informed transforming cell.

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Diapause Induction in Daphnia Requires Two Stimuli

Abstract. *Short-day photoperiods in conjunction with a second stimulus were required to induce sexual reproduction (and diapause) in an autumnally diapausing strain of* Daphnia pulex. *The second stimulus was associated with the unalkalized portion of a small lake and with density of the population in laboratory cultures. Diapause was induced at 12° and 19°C.*

The cladoceran *Daphnia* alternates parthenogenetic with sexual reproduction in a variety of seasonal cycles. Development of the sexually derived embryo is arrested in an early stage, and the arrest is considered a true diapause (*1*). The appearance of sexual reproduction has been explained on the basis of either environmental or intrinsic regulation. In the latter explanation, which arose apparently from studies at a time that coincided with the season of sexual reproduction (*2*), it was assumed that only certain generations and broods were capable of sexual reproduction. The temperature-independent and synchronous initiation of sexual reproduction (and diapause) at different altitudes (*3*) and the simultaneous appearance in lake population and laboratory culture (*4*) were offered in support of intrinsic regulation. Those cases for environmental control were accounted for by the proposal that a period of lability precedes obligatory sexual reproduction. There are ample arguments opposing intrinsic control in the early discussions of reproduction in *Daphnia* (*5*).

Environmental stimuli associated with the reversal from parthenogenesis include density of culture, evaporation of habitat, starvation, low temperature, diet, and metabolic depressants (*6*, *7*). The possibility of photoperiod influence was overlooked, and, without such a possibility, many studies seem not to distinguish regulation in populations which reproduce sexually (initiate diapause) only in autumn from regulation in those which repeat in spring and autumn.

Fortuitous circumstances permit a new hypothesis consistent with the results of earlier studies. In the unlimed (Paul Lake) part of a recently separated lake (Fig. 1), the population of *D. pulex* (and *D. rosea*) initiate sexual reproduction (diapause) in autumn only with considerable precision. No evidence for other than parthenogenesis (nondiapause) was discovered in the other portion (Peter Lake), to which hydrated lime had been added. To account for the precision of onset and the restriction of sexual reproduction to unlimed Paul Lake, the hypothesis proposes a need for two stimuli, one of which could be photoperiod.

The hypothesis was tested with cultures brought into the laboratory and maintained on cell suspensions of *Chlamydomonas reinhardi* Dangeard, Indiana University strain 89, in lake water or synthetic substitute. In the tests of photocontrol, groups of five young *Daphnia* were reared throughout life in glass vials (25 by 95 mm) containing 20 ml of medium. At 2-day intervals the number of released parthenogenetic or sexual (ephippial) broods was counted, and the adults were transferred to fresh medium in which was suspended 1.5×10^5 cells per milliliter. Cycles of light and dark (LD) were regulated by clock-controlled switches; light intensity of approximately 1350 lux was generated by "daylight" fluorescent tubes.

Both populations responded to photoperiod in the laboratory. In the Paul Lake stock, which was studied in sufficient detail, the transitional reversal from parthenogenesis to sexuality was promoted by LD 13.5:10.5 when cultures were maintained at 19°C (Fig.

2). One-half of the broods were sexual (ephippial) at LD 12.75:11.25, and the critical photoperiod was shifted only 15 minutes at an incubation temperature of 12°C. Approximately 6 to 16 LD cycles were necessary before the first sexual brood was released when adults were transferred from a long-day photoperiod.

That duration and not quantity of light is responsible was confirmed in experiments with skeleton photoperiods. Two sets of cultures (Peter Lake stock) were exposed to light for an 8-hour period that was followed by a second exposure of 1.0 hour after 3 or 5 hours of darkness. When the second exposure formed the skeleton of a 12-hour day [8(3)1(12); dark periods in parentheses], sexual broods were induced. In the skeleton of a 14-hour day [8(5)1(10)] all broods were parthenogenetic.

Photoinduction was conditional, however, and the intensity of sexual reproduction was determined by the density of the culture. Densities ranging from 3 to 30 individuals (Peter Lake stock) per 50 ml of medium permitted sexual reproduction to range from 0.0 to 100 percent of the broods in LD 12 : 12 at 19°C (Fig. 3). Crowding failed to induce sexual broods at noninductive photoperiods.

Some observations suggest that sex determination of parthenogenetic eggs is also under the influence of photoperiod. No males were produced in the first week after transfer of cultures (Peter Lake stock) to short-day photoperiods. In the next 3 weeks 48.6 and 53.8 percent of the young born were males at LD 12:12 and 13:11, respectively. At LD 14:10, 5.9 percent of the young were males, and at 16:8 no males were produced.

Results of the laboratory experiments corroborate field observations that suggest a need for two stimuli to induce sexual reproduction in populations of *Daphnia* which reproduce sexually only in autumn. Excluded from these conclusions are those races of *Daphnia* which enter diapause under long-day photoperiods and those which overwinter as adults, although the former could result from an overriding of photocontrol and the latter, from an absent or deficient second stimulus in the environment. The probability that races of *Daphnia* differ in susceptibility to the stimuli precludes further analysis without additional experimentation.

The nature of the second stimulus, other than its association with density of the culture, is unknown. Both lack of food, since the quantity was standardized regardless of density, and the accumulation of metabolic products are implicated. The latter would be consistent with the conclusions of Banta and co-workers (*7*) for Cladocera and with the control of sexual reproduction in other animals (*8*).

This report relates the first instance known to us in which photoinduced diapause in the wild (albeit manipulated) condition may be deferred in the absence of a second stimulus. In the laboratory, temperature (*9*) and diet (*10*) are known to affect intensity of diapause in terrestrial arthropods but only in ecologically inconsistent en-

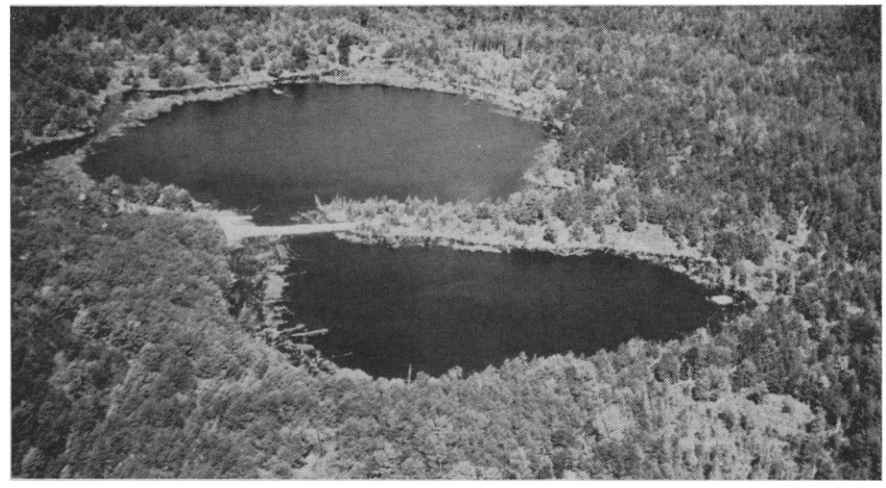

Fig. 1. Lakes Paul (foreground; 1.2 ha) and Peter (2.4 ha) in Michigan's Upper Peninsula (T45N, R8E, S36). Peter Lake has been treated with hydrated lime (2500 kg/ha) (*12*).

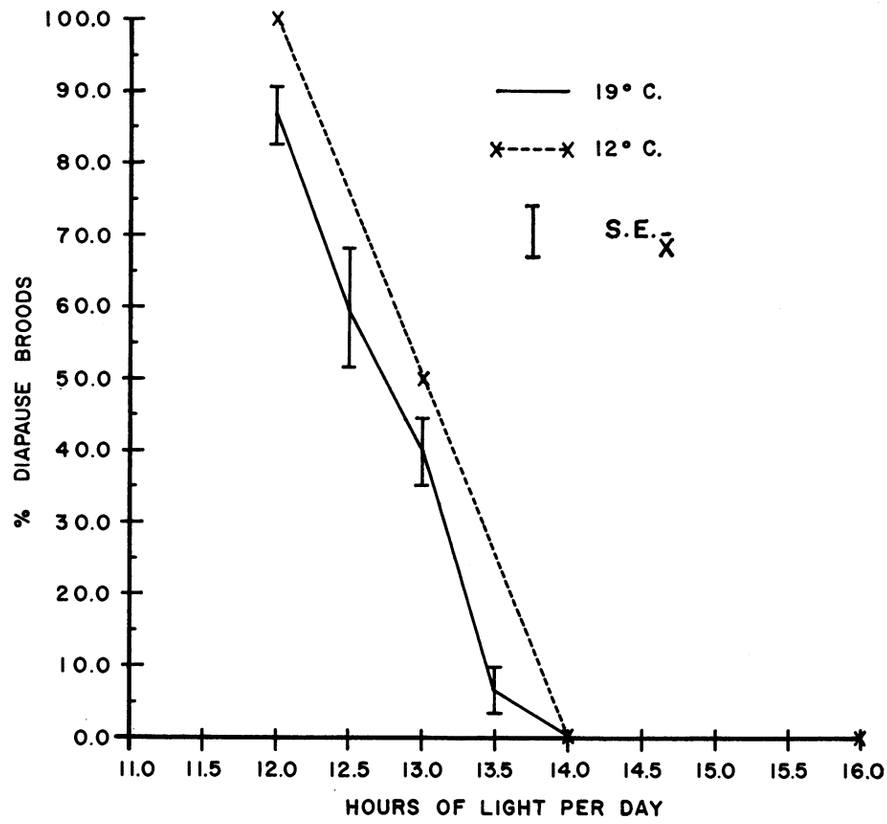

Fig. 2. Influence of photoperiod on sexual reproduction in *Daphnia pulex* (Paul Lake stock) as measured by the percentage of sexual (diapause) broods. Each replicate consists of five individuals per 20 ml of medium cultured throughout the experiment (30 days) at photoperiod indicated.

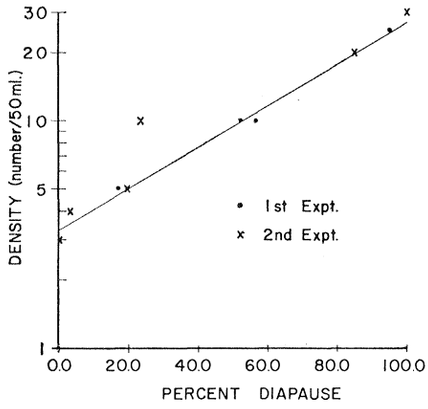

Fig. 3. Intensity of sexual reproduction in *Daphnia pulex* (Peter Lake stock) as a function of population density. Intensity of sexual reproduction (diapause) increases with log of density. The cultures were maintained at 19.0°C and at LD 12:12. The adults were transferred every other day into 50 ml of medium containing a standard concentration of food.

vironments. Unlike the terrestrial, the aquatic environment of north temperate latitudes affords adequate heat to permit a motile (adult) stage to overwinter (*11*).

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12. The lakes were separated in 1951 by A. D. Hasler for studies of lake alkalization. Observations of the time of sexual reproduction were made in six different years, beginning in 1954. A description of the effects of alkalization may be found in R. G. Stross and A. D. Hasler, *Limnol. Oceanogr.* **5**, 265 (1960).

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Adrenaline Synthesis: Control by the Pituitary Gland and Adrenal Glucocorticoids

Abstract. *The activity of phenylethanolamine-N-methyl transferase, an enzyme that synthesizes adrenaline from noradrenaline in the adrenal medulla, is markedly depressed following hypophysectomy. Enzyme activity is restored to normal after administration of ACTH or the potent glucocorticoid, dexamethasone. Thus the biosynthesis of adrenaline in the adrenal medulla appears to be regulated by the pituitary-adrenocortical system.*

The final step in the biosynthesis of adrenaline involves the transfer of a methyl group from *S*-adenosylmethionine to the amine nitrogen of noradrenaline (*1*). This process is catalyzed by an enzyme, phenylethanolamine-*N*-methyl transferase (PNMT), which is highly localized in the adrenal medulla of mammals (*2*). Although low levels of activity of this enzyme have been detected in heart and brain, it is likely that almost all of the adrenaline in the circulation is derived from catecholamine synthesized within the adrenal medulla (*3*).

Little is known about the factors which control the formation of adrenaline in vivo. In species in which the adrenal medulla is not surrounded by adrenocortical tissue, the catecholamine content of the medulla is almost exclusively noradrenaline (*4*). On the basis of this observation, it has been suggested that the mammalian adrenal cortex secretes a factor which influences the methylation of noradrenaline (*4*). We now show that the activity of the adrenaline-forming enzyme, PNMT, in adrenal medulla of the rat is regulated by pituitary adrenocorticotropic hormone (ACTH) and by adrenal glucocorticoids.

In our experiments, the PNMT activity and catecholamine content of the adrenal glands were measured in normal rats and in animals subjected to hypophysectomy or treatment with various hormone preparations. Groups of Sprague-Dawley female rats (*5*) weighing 160 to 200 g were killed after 6 days of treatment with hormone or placebo. Both adrenals were rapidly dissected free of fat, weighed, and homogenized in 2 ml of chilled isotonic potassium chloride solution. The homogenate was centrifuged at 100,000*g* for 30 minutes. A portion of the supernatant fluid was assayed for PNMT activity by a modification of a method described before (*2*, *6*). The remainder of the whole adrenal homogenate was diluted with an equal volume of 2*N* acetic acid and shaken vigorously in the cold. A portion of adrenal tissue was assayed for adrenaline and noradrenaline by a modification of the method of von Euler and Lishajko (*7*).

Hypophysectomy was associated with a marked reduction in the activity of PNMT in the rat adrenal (Table 1). This fall was of greater magnitude than the decrease in adrenal weight (which is due primarily to atrophy of adrenocortical tissue.) The amount of adrenaline contained in both adrenals was reduced by hypophysectomy, as was the percentage of the total adrenal catecholamine represented by adrenaline (Table 1). The fall in adrenaline content could represent changes in storage or release of the amine. However, it is also consistent with a decrease in the rate of synthesis of adrenaline, brought about by a decline in PNMT activity. When hypophysectomized rats were given daily injections of ACTH for 6 days, the total activity of PNMT in adrenal tissue increased threefold, returning almost to normal levels (Table 1). The fraction of adrenal catecholamine represented by adrenaline showed a similar response. Hökfelt has also observed an increase in the content of adrenaline in adrenals of hypophysectomized animals treated with ACTH (*8*).

There are three possible mechanisms whereby ACTH could have enhanced the enzymic methylation of noradrenaline in the hypophysectomized rat. (i) There could be a methylating enzyme in the adrenal cortex which responds directly to ACTH. (ii) ACTH could act on PNMT in the adrenal medulla. (iii) ACTH could act indirectly, by enhancing the synthesis of glucocorticoids in the adrenal cortex and their delivery to the medulla. To test the first hypothesis, the adrenals of 10 normal rats were divided into medulla and cortex, and each component was assayed for PNMT. Less than 10 percent of the adrenaline-forming activity was present in cortical tissues.

To determine whether ACTH acted directly on the adrenal medulla, two experiments were performed: First, normal rats were treated for 6 days with methopyrapone (1 mg daily). This agent depresses the biosynthesis of adrenal glucocorticoids by inhibiting the 11-β-hydroxylase enzyme in the adrenal cortex (*9*). This inhibition results in a fall in blood glucocorticoid