carbonyl compounds are easily detected as orange spots when the bugs are placed on filter papers saturated with 2,4-dinitrophenylhydrazine. The ejection can be either bilateral or unilateral. Unilateral ejection was most commonly observed when the bugs were approached by imported fire ant workers (*Solenopsis saevissima* v. *richteri* Forel). Ants which were exposed to the spray rapidly moved away. This would seem to support the belief that the odoriferous secretions of the pentatomids are at least partially protective.

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Use of Cytoplasmic Male-Sterility in Making Interspecific Crosses in Allium

Abstract. Cytoplasmic male-sterile plants of *Allium cepa* were used in making interspecific crosses with *A. fistulosum.* Some inbred lines of *A. cepa* produce more seed than others. Other *Allium* species could also be used as the pollen parent.

In plant breeding hand emasculation is often slow and painstaking; the results are somewhat disappointing and the number of F_1 progeny is limited. The cytoplasmic male-sterile character in *Allium cepa* L., as reported by Jones and Clarke (*1*), is extremely useful in crossing *A. cepa* and *A. fistulosum* L.

Eight cytoplasmic male-sterile inbred lines of *A. cepa,* each represented by 10 mother bulbs, were placed in an insect-proof isolation cage 3½ by 6 by 6 feet. Though *A. cepa* bloomed much later than *A. fistulosum,* no particular difficulty was encountered. By growing several thousand *A. fistulosum* plants, a sufficient number of late-flowering umbels were obtained. The seedstalks, with stems as long as possible, were cut and placed in a container of water to which 1 part of copper per million in the form of copper sulfate was added to prevent growth of fungi and algae. The container of flowers of *A. fistulosum* was then placed in the cage with the *A. cepa* inbred lines. Honey bees (approximately 3 pounds of workers with a queen, brood, comb, and so forth) were used as the pollinators.

The inbred lines used and the number of seeds from each inbred line are given in Table 1. Of course, the difference in bloom time could account for some but probably not all of the difference noted. I feel that some inbred lines will cross more readily with *A. fistulosum,* although sufficient data are not available for a definite statement. The well-known constancy of bees in pollinating a particular species, strain, or even individual plant, or their preference for plants with high sugar levels in the nectar as reviewed by Grant (*2*) was not a factor in the pollination of the material in this report. The bees were confined to a small volume and were not free to forage. Food was not too plentiful within the cage. The bees visited each and every plant without preference for one or the other. Some of the F_1 progeny were male-fertile, others male-sterile. Ratios were not determined.

The characteristics of *A. fistulosum* are sufficiently distinct from those of *A. cepa* that the two species are readily identified. The hybrid between the two species is intermediate in character. Plants grown from the seeds reported in Table 1 were hybrids between the two species. Emsweller and Jones (*3*) have described the interspecific hybrid.

This system of crossing eliminates emasculation, reduces possible contamination, increases the chance of a cross, and produces a greater number of seeds. When single umbels are being crossed, houseflies or blue-green bottle flies can be used as pollinators. A male-sterile umbel of *A. cepa* can be enclosed in a small cage with the male-fertile umbel of *A. fistulosum.*

Though only a few seeds were produced, they were adequate to grow out the F_1 generation. The F_1 interspecific hybrids produced in the foregoing manner may be either male-fertile or male-sterile. Male-fertile plants may be used as the pollen parents in a backcrossing program with male-sterile *A. cepa* as the recurrent female parent. Male-sterile F_1 interspecific hybrids can be used as the female parent with a male-fertile *A. cepa* as the pollen parent.

Table 1. Number of seeds produced on eight male-sterile *A. cepa* inbred lines pollinated by *A. fistulosum* with honey bees in an insect-proof isolation cage, Parma, Idaho, 1955.

Inbred source	Pedigree	No. of seeds
Early yellow globe	B 2108 A	30
Early yellow globe	B 2117 A	40
Brigham yellow globe	B 2190 A	200
Brigham yellow globe	B 2207 A	75
Brigham yellow globe	B 2217 A	30
Brigham yellow globe	B 2218 A	55
Brigham yellow globe	B 2267 A	40
Yellow sweet Spanish	B 12132 A	20

Although only *A. cepa* was crossed to *A. fistulosum* by this method, other *Allium* species could be used as the pollen parent. The system is simple and effective in making interspecific as well as intraspecific crosses in the *Allium* species, in which a male-sterile *A. cepa* can be used as the seed parent (*4*).

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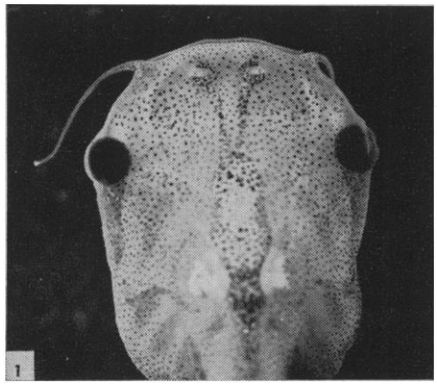
Pineal Regulation of the Body Lightening Reaction in Amphibian Larvae

Abstract. Body pallor due to contraction of both deep and integumental melanophores occurs when either blinded or normal *Xenopus laevis* and other amphibian larvae are placed in the dark. The reaction is abolished by pinealectomy, but is induced by administration of pineal hormones. It is suggested that the normal body lightening reaction is mediated by the pineal gland.

It has been known for many years that due to melanophore contraction amphibian larvae become pale when subjected to darkness for periods of a few hours (*1–3*). The mechanism of this lightening reaction, however, remains unexplained and our understanding of it has been further complicated by observation that the phenomenon is not abolished in blinded larvae (*2*). With this in mind and as a result of the discovery that the tail darkening reaction of *Xenopus laevis* is due to a direct effect of light on tail melanophores (*4*), it was suggested that a similar photochemical mechanism might mediate the body lightening reaction (*3*). In the course



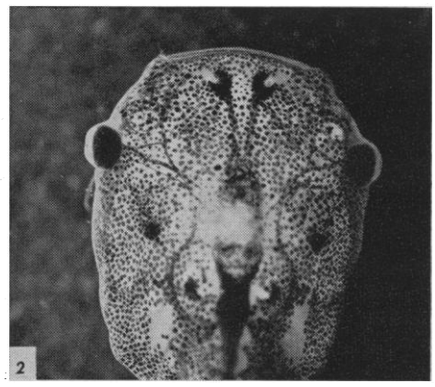


Fig. 1 (left). Normal *Xenopus* larva at the height of body lightening after 60 minutes in the dark. Note the extreme contraction of melanophores in the skin and on blood vessels and nerves. The paired thymus glands which are usually quite obvious because of their heavy pigmentation can hardly be seen. Fig. 2 (right). Pinealectomized *Xenopus* larva after 60 minutes in the dark. No melanophore contraction has occurred and as a result the thymus glands, optic nerves and various blood vessels are clearly discernible. Scar indicates site of cautery.

of an investigation of this possibility, an alternative hypothesis involving the pineal gland was developed because of the well-known visual associations of the pineal complex (*5*) and the frequently reported melanophore contracting activity of pineal extracts (*6*). In the investigation described in this report, both of these hypotheses were evaluated experimentally, and a mechanism is proposed to explain the normal regulation of the body lightening reaction.

When normal or recently blinded *Xenopus* larvae are placed in the dark, melanophore contraction begins in about 15 minutes, at normal laboratory temperatures (about 23°C), and maximum pallor occurs (Fig. 1) in approximately 30 minutes. After the animals are returned to normal illumination, re-expansion of body melanophores of these lightened tadpoles proceeds slowly, requiring 45 to 60 minutes for the restoration of normal pigmentation. Similar temporal factors are observed in the body lightening reaction displayed by larvae of several other amphibians, including various species of *Rana* and *Ambystoma*.

The time intervals involved in these data strongly refute the possibility of a photochemical effector for body lightening; for if such a system is involved, as it is in the tail melanophores of *Xenopus*, one should expect body lightening to occur relatively slowly during the interval in the dark and to disappear very soon after larvae are returned to light. Further evidence against the possibility that body melanophores are directly sensitive to changes in illumination is derived from a series of experiments done in vitro. The results of these experiments indicate that melanophore contraction does not occur in the skins of the dorsal surface of *Xenopus* larvae which are maintained in various physiological salt solutions and subjected to periods of darkness. That these melanophores retain physiological activity is demonstrated by their response to various melanocyte-stimulating hormone preparations (*7*) added to the medium.

The time factors involved during the onset and termination of the body lightening reaction suggest that small amounts of a "hormone" might be released when tadpoles are placed in the dark and that release of this "hormone" ceases when they are returned to the light. This would explain why the reaction starts as quickly as it does. It seems logical to assume that during the subsequent 45 minutes required for redarkening, normal metabolic processes reduce the amount of "hormone" to an ineffective level. At this point one of our unpublished experiments was recalled in which larvae of *Ambystoma opacum, Rana pipiens,* and *Xenopus laevis* were immersed in dilute solutions of melatonin (N-acetyl-5-methoxytryptamine), a potent, direct acting, melanophore contracting compound isolated by Lerner (*8*) from the pineal glands of cattle. Body lightening induced in larvae by this compound is identical to that which is seen when tadpoles are placed in the dark (Fig. 1). Deep melanophores on blood vessels, nerves, and various organs, as well as those in the integument, contract markedly. Furthermore, melatonin at a concentration of 0.01 mg/ml of aquarium water elicits body lightening, but does not inhibit the tail darkening reaction. This response seems identical to that which occurs when *Xenopus* larvae are placed in the dark. Higher concentrations of melatonin completely abolish tail darkening (*9*).

Altogether, these observations seemed to implicate the pineal gland, and as a result the body lightening reaction was investigated in over 25 *Xenopus* larvae, at various stages, which had been deprived of their pineal glands. "Pinealectomy" was performed by cauterizing the pineal area of the diencephalic roof with either a hot needle or a cold cautery apparatus. Larvae with cauterized parts of the optic tectum or olfactory lobes served as sham-operated controls. Blinded, sham-operated, or normal larvae displayed typical body lightening when placed in the dark. Pinealectomized larvae consistently displayed no melanophore contraction under these conditions (Fig. 2), and their tails were even darker than those of the control groups.

On the basis of all the data presented in this report, it is proposed that the following mechanism operates in the normal regulation of the body lightening reaction observed in the larvae of many amphibian species. When larvae are placed in the dark, the lack of either sufficient quantities or appropriate wavelengths of light almost immediately stimulates the pineal gland to secrete small amounts of melatonin or a similar substance. This compound, which is active at very low concentrations (*10*), overrides melanophore expansion induced by hypophyseal chromatotrophic hormone and causes body melanophores to contract, giving rise to the body lightening reaction. When larvae are returned to normal illumination, the pineal is affected and it ceases to release melatonin. Gradually, normal metabolic processes reduce the quantity of circulating hormone to a level below that necessary for melanophore contraction. Subsequently, redarkening occurs. The fact that the latter requires only about 45 minutes, together with the observation that tail darkening is hardly affected, indicates that only small quantities of melatonin are released during the occurrence of the normal body lightening reaction.

The mechanism proposed in this report represents an integration of two events, light reception and melanophore contraction. Both of these have often been implicated in studies of pineal complex, but to my knowledge the present investigation is one of the few demonstrations of their association in normal melanophore reactions (*11*).

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Experimental Study of Teratogenic Effect of Emotional Stress in Rats

Abstract. The teratogenic effect of audio-visual and immobilization stress was studied in rats. Groups of 15 animals were subjected from the 9th to the 12th day of pregnancy to one or the other of these types of stress or to stress combined with administration of vitamin A. It was shown that the stresses alone had no effect on congenital malformations. Immobilization stress seemed to potentiate the teratogenic effect of vitamin A.

Rats of the Holtzman strain, ranging in weight from 250 to 320 g, were used in the experiment reported here (*1*). The female animals were kept together with males. Vaginal smears were taken every night and examined for spermatozoa. The day on which spermatozoa were found was regarded as the first day of pregnancy. The pregnant rats were divided at random into six groups with 15 animals in each group. The groups were treated as follows. Group 1 consisted of control animals. Rats in group 2 were subjected to intermittent ringing of bells and flashing of light (*2*) from the 9th to the 12th day of pregnancy for 6 hours daily. Rats in group 3 were subjected to immobilization, as described by Renaud (*3*), for 3 hours on the 9th day and for 4 hours from the 10th to the 12th day of pregnancy. Each rat in group 4 received, by intubation, 15,000 international units of vitamin A in oily suspension (*4*) daily from the 8th to the 12th day of pregnancy. Rats in group 5 were also given the vitamin A and, in addition, were subjected to audio-visual stress, like those in group 2. Rats in group 6 received the vitamin A and, in addition, were subjected to immobilization, like those in group 3.

All animals were allowed to eat Purina laboratory chow ad libitum and were also fed raw potatoes and dry bread three times a week. The animals were killed on the 20th day of pregnancy. The young were removed, weighed, and preserved in 10-percent Formalin until they had been examined macroscopically. Deformities of the brain and calvaria as well as cleft palate were recorded.

The results are shown in Table 1. No congenital malformations were found in young of the control group or of the groups which were subjected to stress only (groups 2 and 3). In the group fed vitamin A but not subjected to stress (group 4) there was a low incidence of malformations; these were confined to about one-third of the litters. About the same proportion of malformations occurred in the group in which feeding of vitamin A was accompanied by audio-visual stress (group 5). In the young of rats that had been fed vitamin A and had also been immobilized (group 6), the percentage of cleft palates was eight times as high as in the young of those that had only been given the vitamin, and the percentage of young with some malformation was about five times as high. The percentage of resorbed embryos in group 6 was relatively high—a fact which explains the lower number of young per litter in the group. This may also explain the small number of young with deformities of brain and calvaria in this group, since it is known that embryos with severe malformations tend to die and are then resorbed.

Animal experiments have shown that administration of cortisone during pregnancy increases the teratogenic effect of vitamin A hypervitaminosis in rats (*5*). Cortisone has also been found to increase the incidence of cleft palate in mice of a genetic strain in which there is normally a low incidence of cleft palate (*6*). Since stimuli causing nervous excitement are known to increase adrenal cortical secretion (*7*), a similar, teratogenic effect might be expected as a result of emotional stress.

Although the audio-visual stress had some effect on the behavior of the animals in our experiment, causing them to hide under each other and to wash their faces (*8*), it did not increase the frequency of vitamin-A induced deformities. Possibly it was not severe enough. Immobilization is a severe stress in rats; it causes a typical alarm reaction, with enlargement of the adrenals within a few hours (*3, 9*). Its severity, even if applied for 3 to 4 hours on 4 days only, is shown by the fact that a vaginal bleeding with coagula was observed in four animals during immobilization. The bleeding appeared on the 11th or 12th day of pregnancy and almost certainly was a sign of abortion. It differed radically from the small physiological uterine bleeding which is a characteristic sign of pregnancy in rats and appears usually on the 14th day of pregnancy (*10*). Since the uteri of the animals with observed bleeding looked like the uteri of the other nonpregnant animals, it is quite probable that immobilization had caused a miscarriage in most of the nonpregnant animals of groups 3 and 6.

Immobilization alone did not cause any malformations, but it seemed to increase the teratogenic effects of vitamin A. This result is similar to the findings of Millen and Woollam (*5*), that cortisone administration increases the teratogenic effect of vitamin A hypervitaminosis in rats but does not itself cause malformations. Immobilization has been called a pure emotional stimulus (*3, 9*), but this can be argued, of course, and the same authors have also called it a "neuromuscular exer-

Table 1. Effect on congenital malformations in the offspring of rats of audio-visual stress, immobilization stress, and vitamin A plus stress. Figures in parentheses, percentages of total.

Item	Controls	Stress		Vitamin A	Vitamin A plus stress	
		Audio-visual	Immobilization		Audio-visual	Immobilization
Rats with spermatozoa in vaginal smear (No.)	15	15	15	15	15	15
Mean weight of rats on 1st day of pregnancy ± standard error (g)	266.3 ±6.25	269.0 ±8.51	262.3 ±6.87	272.3 ±6.75	271.2 ±6.12	270.7 ±4.76
Rats with young (No.)	14	13	8	14	14	5
Rats with vaginal bleeding (observed abortions) (No.)			2			2
Totally resorbed embryos (No.)		7	3	2	1	9
Young (total No.)	182	152	102	178	162	49
Mean weight of young (g)	2.40	2.47	2.45	2.46	2.65	2.41
Young per litter (No.)	13.0	11.7	12.8	12.7	11.6	9.4
Litters with deformities (No.)				5	5	3
Young with deformities (total No.)				13 (7.3)	7 (4.3)	18 (36.7)
Young with deformities of brain and calvaria (No.)				11 (6.2)	3 (1.9)	1 (2)
Young with cleft palate (No.)				8 (4.5)	4 (2.5)	18 (36.7)