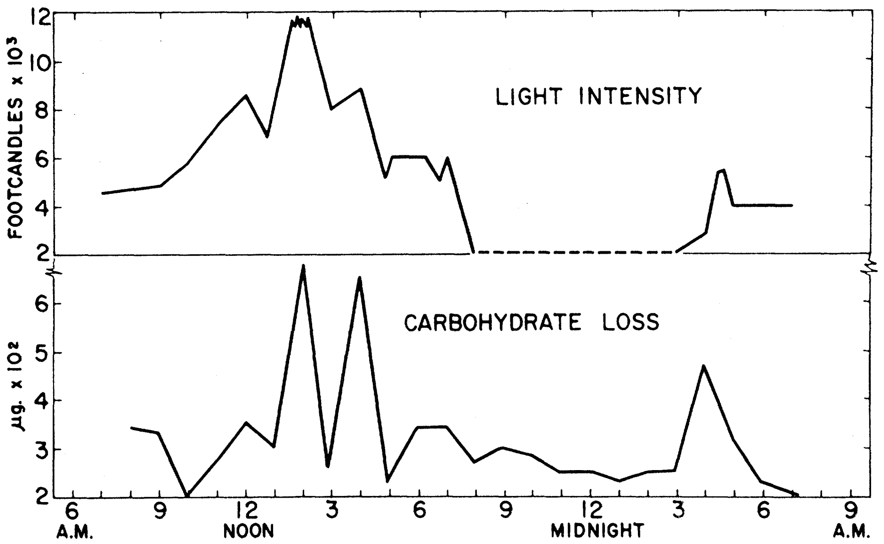

Fig. 1. Loss of carbohydrates from bean leaves by leaching with distilled water as related to light intensity. (Top) Hourly variation of light intensity in foot candles; (bottom) average hourly carbohydrate loss in micrograms per leaf.

be leached from leaves (*1, 3*). During the course of leaching studies at our laboratory, an interesting relationship between carbohydrate loss and light intensity has been noted and is herein reported (*4*).

Bean seeds (*Phaseolus vulgaris,* var. Contender) were germinated in sand, and the seedlings were transferred to aerated cultures containing half the nutrient intensity of Hoagland's standard solution (*5*). After 2 weeks, an entire primary leaf, while still attached to the intact plant, was immersed in 130 ml of distilled water in a flat vessel. Leaves on six plants were used for replication. At hourly intervals for 24 consecutive hours, including the natural daylight and dark periods, the solutions in the vessels were drawn off and replaced with fresh distilled water. The solutions containing the leaf leachate were evaporated on a steam bath to a standard volume of 10 ml and analyzed colorimetrically for carbohydrates (*6*). The plants were harvested at the end of the leaching period; the leaves which had been leached were removed, and dry weights were determined. Light intensities were recorded during the experimental period.

The results of the study are presented in Fig. 1. The upper half of the graph shows hourly variations of light intensity in foot candles, and the lower half shows the hourly losses of carbohydrates in micrograms per leaf. The two curves are remarkably similar, showing an apparent direct relationship between light intensity and the leaching of carbohydrates. It will be observed that greatest removal of carbohydrates occurred during the periods of highest light intensity. Only small losses occurred during darkness. The total loss of carbohydrates during the 24-hour period was 7.5 mg per leaf, which was 4.8 percent of the dry-weight equivalent of the leached leaves. Variation among the replications was slight in both total and hourly losses. The principal carbohydrate leached from bean leaves under similar conditions has been identified as a galactan (*1*).

To substantiate further the relationship between carbohydrate loss and light intensity, two variations were introduced into the afore-described experimental procedure: (i) the leaf being leached was left exposed to light, and the remainder of the plant was covered with a black cloth; and (ii) the leaf being leached was covered with a black cloth, and the remainder of the plant was exposed to light.

In general, the results were similar to those reported earlier in this paper. When the leaf was left exposed to light (variation i), loss of carbohydrates paralled the intensity of light; and when the leaf was covered with the cloth (variation ii), carbohydrate losses were constantly low and did not fluctuate with the changes of light intensity. Further, although the temperature of the leaching solution and the covered leaf (variation ii) rose somewhat during the hourly intervals, no relationship between these rises and the carbohydrate loss could be determined. This tends to show that the light intensity, and not the temperature increase, was the factor associated with carbohydrate loss from the leaves.

Two hypotheses may be suggested to explain these phenomena. First, increased solar radiation stimulates the photosynthetic activity of the leaf. Since newly elaborated carbohydrates are readily water soluble, they are in a condition to be easily leached immediately after manufacture. Second, the mechanism of carbohydrate removal may be affected. There is evidence that the number of plasmodesmal connections from the cells to the leaf surface increases in the light (*7*). The plasmodesmata may aid in establishing a pathway for carbohydrates to be leached from the leaf.

Varying amounts and intensities of rainfall occur in different parts of the world and at different seasons of the year. In addition, many modern agricultural practices—for example, overhead irrigation, mist propagation, syringing, and spraying—involve the application of aqueous solutions to aboveground parts of plants. It has been shown that more than 400 kg of carbohydrates per acre can be removed by rain from the foliage of apple trees during a single growing season (*3*). The close relationship between light intensity and carbohydrate losses by leaching and the significance of these losses, especially at critical times in plant development, await further evaluation.

H. B. Tukey, Jr.
S. H. Wittwer
H. B. Tukey
Department of Horticulture, Michigan State University, East Lansing

References and Notes

1. W. G. Long, D. V. Sweet, H. B. Tukey, *Science* 123, 1039 (1956).
2. M. G. Mes, *S. African J. Sci.* 50, 167 (1954); H. B. Tukey, Jr., S. H. Wittwer, H. B. Tukey, *Abstr. Am. Soc. Hort. Sci. Ann. Meeting,* Storrs, Conn. (1956).
3. S. Dalbro, *Proc. 14th Intern. Hort. Congr.,* in press.
4. This investigation was supported by contract No. AT-(11-1)-159 of the U.S. Atomic Energy Commission, Biological and Medical Division. This is journal article No. 2034 of the Michigan State Agricultural Experiment Station.
5. D. R. Hoagland and D. I. Arnon, *Calif. Agr. Exp. Sta. Circ.* 347 (1950).
6. E. Lunt and D. Sutcliffe, *Biochem. J.* (*London*) 50, 122 (1953).
7. P. Lambertz, *Planta* 44, 147 (1954).

6 May 1957

Thyroxine Effect on Melanophore Contraction in Xenopus laevis

While working with lathyrism in the South African toad, *Xenopus laevis* Daudin, we observed that tadpoles became blanched when they were treated with thyroxine solution. A series of experiments was then designed to investigate the role of thyroxine in the color control mechanism of this species (*1*).

It is well known that in both adult and larval *Xenopus,* removal of eyes results in expansion of the epidermal melanophores (*2*). Figure 1*a* illustrates a skin preparation from a newly blinded tadpole showing the expanded melanophores. When such animals are placed in L-thyroxine-Na solution of various concentrations, they become pale within an hour through contraction of the melanophores (Fig. 1*b*). The minimum effective dose producing this condition is about 1 mg/lit. Blanching is obtained more rapidly if a thyroxine solution is injected into the body cavity. The pale condition may be attained within 15 minutes, and the minimum effective dose is found to be 0.1 μg per tadpole. This blanching effect is reversible. When the treated tadpoles are returned to aquarium water, the melanophores slowly expand and regain their original condition.

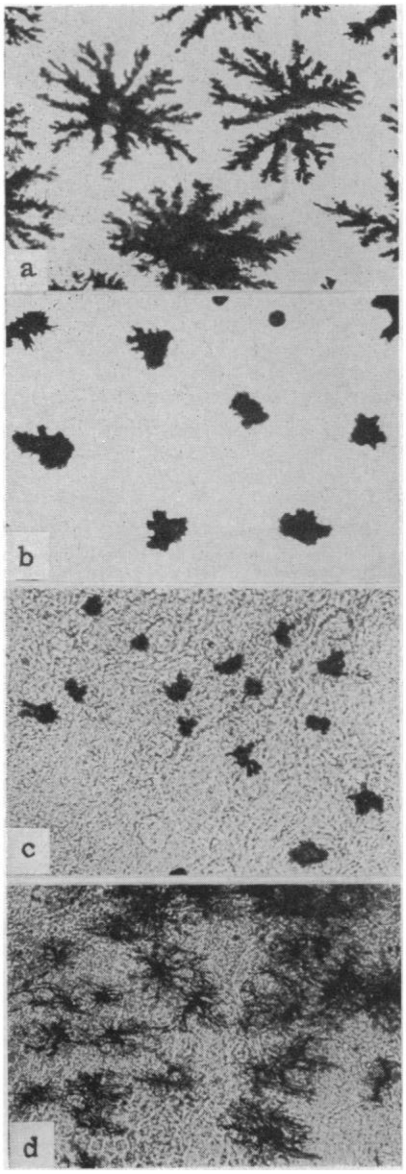

Fig. 1. (*a*) Newly blinded tadpole with skin removed from head region in front of the brain (× 90). (*b*) Same preparation as in (*a*), except that tadpole was treated with L-thyroxine at a concentration of 2 mg/lit. (*c*) Web preparation from blinded adult that had received 1 mg of L-thyroxine (× 100). (*d*) Same preparation as in (*c*) except that the blinded adult received 5 mg of atropine in addition to thyroxine, showing that expansion of melanophores is not affected (× 100).

The adult animals used in the following experiments were blinded females of a body weight of about 120 g. L-Thyroxine-Na was administered by intraperitoneal injection. The minimum effective dose for such mature animals is 0.1 mg/100 g of body weight. The time lapse between injection and color response is the same as it is in tadpoles, about 15 minutes.

The question of whether the thyroxine effect on melanophores is a direct one or is mediated through some intermediate route such as a hormonal or neurohumoral mechanism was answered in the following experiments. When pieces of isolated skin of tadpoles or web from blinded adult animals were immersed in distilled water (*3*) containing thyroxine at a concentration of 2 mg/lit, the epidermal melanophores failed to contract. The result indicates that an indirect mechanism is involved. This is further demonstrated by the following experiment. Both normal tadpoles and adults were first darkened by pretreatment with injection of melanophore-stimulating hormone. Thyroxine was then given to these animals either by placing the animals in solution, as in the case of tadpoles, or by injection, as in the case of adults. Regardless of the treatments, all animals in both groups remained dark. Evidently the melanophores are not affected by the thyroxine that is administered under these conditions.

The possibility of an inhibitory mechanism by thyroxine on release of melanophore-stimulating hormone from the pars intermedia is also ruled out by the fact that a larva with its transplanted adult hypothesis in the body cavity does not react to thyroxine treatment. The animals showed a slight pale tint but were not blanched, in contrast to the experimental controls. This result points out the possibility of the participation of a neurohumoral mechanism of the hypothalamus. For demonstration of the presence of such a mechanism, both dibenamine (antiadrenergic) and atropine (anticholinergic) were used. Four blinded adult females were injected with 5 mg of dibenamine each and another four animals with the same amount of atropine. One hour later, 1 mg of thyroxine was given to each of the eight animals. Another four animals served as controls, and each received 1 mg of thyroxine only. The animals that received dibenamine and those that received thyroxine become blanched (Fig. 1*c*), while the animals that received atropine remained dark, like the untreated controls (Fig. 1*d*). This demonstrates clearly that the effect of thyroxine on the color control mechanism is an indirect one, involving a neurohumor of cholinergic nature which in turn controls the release of melanophore-stimulating hormone from the pars intermedia. Injection of acetylcholine also brings out the contraction of melanophores. But the dosage required is relatively high (5 mg per adult animal), probably because of the rapid destruction of this substance in the body by the presence of cholinesterase.

Since L-triiodothyronine fails to affect color change in this species even with a dosage 10 times greater than that of thyroxine used, it appears that the thyroxine effect is highly specific. Our unpublished results show that thyroxine may also be involved in initiating ovulation through stimulation of the hypothalamus (*4*). It seems that thyroxine may play a more important role in endocrine physiology than we know at the present time.

CHIH YE CHANG
Department of Zoology, State University of Iowa, Iowa City

References and Notes

1. This work was aided by a grant from the Rockefeller Foundation. I am also indebted to I. V. Ponseti, E. G. Gross, and Armour Research Laboratories for gifts of L-thyroxine-Na, dibenamine hydrochloride, and melanophore-stimulating hormone, respectively.
2. For early literature on pigmentation, see G. H. Parker, *Animal Color Changes and Their Neurohumours* (Cambridge Univ. Press, Cambridge, England, 1948). For recent discussion on *Xenopus* color changes, see A. C. J. Burger's thesis, ***Investigations into the Action of Certain Hormones and Other Substances on the Melanophores of the South African Toad,*** Xenopus laevis (G. W. Van Der Wiel, Arnhem, Netherlands, 1956).
3. Amphibian Ringer's solution was found to be unsuitable because it produced a nonspecific temporary contraction of melanophores.
4. C. Y. Chang and E. Witschi, unpublished data.

24 April 1957

Control of Etiolated Bean Leaf-Disk Expansion by Gibberellins and Adenine

The action spectrum for promotion of etiolated leaf expansion by red light (6500 A) and the reversal of this effect by far red light (7350 A) (*1*) is analogous to the one controlling flowering (*2*). The mechanism involved in this biochemical reaction is unknown, although research directed toward its understanding has revealed several important points. Among these points is that the response depends on the quality of light given last. The promotion of leaf expansion by cobalt (*3*) and kinins (*4*) is essentially additive to the promotion by red light, and, in addition, these substances appear to be capable of modifying the response to far red light (*5*).

In attempts to elucidate still further the interaction of kinins with light in controlling leaf expansion, approximately