centrations, 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.

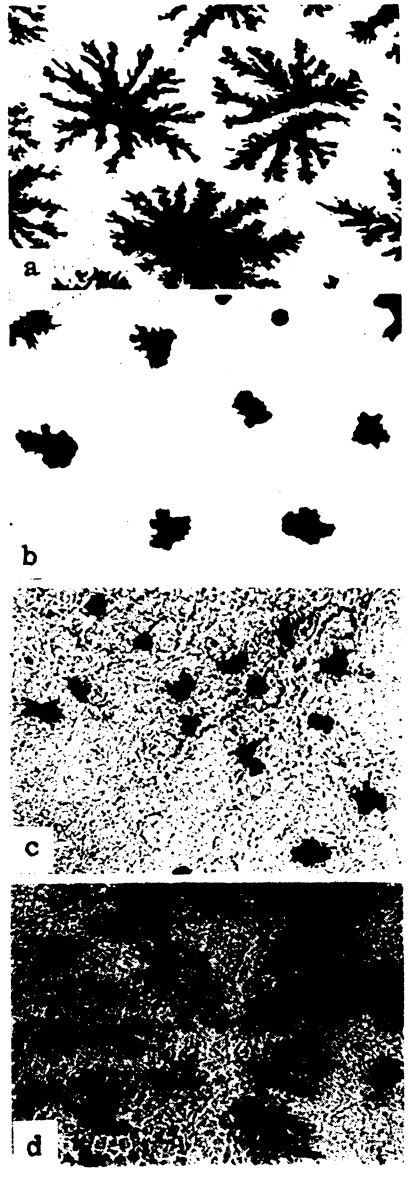

Fig. 1. (*a*) Newly blinded tadpole with skin removed from head region in front of the brain (× 90). (*b*) Same preparation as in (*a*), except that tadpole was treated with L-thyroxine at a concentration of 2 mg/lit. (*c*) Web preparation from blinded adult that had received 1 mg of L-thyroxine (× 100). (*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.

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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).
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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

60 additional kinins have been examined (*6*). Although most of these show a promotion which is simply additive to the promotion by red light, the experiments cited in the report (*7*) show that adenine and some closely related analogs produce an appreciable amount of growth that is equal in all light conditions and in darkness.

The observation that the gibberellins alter the red-light-induced inhibition of elongation growth (*8, 9*) prompted the examination of these substances for their ability to control etiolated bean leaf disk expansion. The experiments reported here show that the gibberellins (*10*) promote the expansion of etiolated leaves and that this promotion is additive to that induced by red light.

Seed of Burpee Dwarf Stringless Green Pod beans were soaked for three hours, treated with Ceresan, and then grown in river-washed sand contained in flats in a dark room maintained at 25 ± 1°C until the leaves were approximately 2 to 2½ cm² in area. Disks 5 mm in diameter were taken from the leaves and placed in petri dishes on filter paper moistened with 5 ml of basal solution, where they received chemical and light treatments in a manner indicated previously (*4*).

Table 1 indicates the magnitude of increase induced by the various substances after 48 hours of growth. The most important result of these experiments appears to be that the promotive action of adenine and thiopurinesuccinic acid is independent of the quality of light to which the disks are exposed, and in fact independent of whether light is given at all. Our unpublished experiments show that this type of response is exhibited only by compounds closely related to adenine (*6*). The response caused by adenine should be compared with that given by leaf disks grown in basal medium or in a medium containing cobalt. In the latter two cases, both the red-light effect and its reversal by far red light are observed. Although Bonner and Haagen-Smit (*11*) showed that adenine is active in controlling mesophyll development, they never obtained as great a difference between controls and treated leaves as that reported here. The present results suggest that adenine or some closely related compound may be the final product of the red-light-mediated reaction in leaf-disk expansion since all of our results to date show that adenine can completely replace the red-light requirement in this system. As the results reported here show, adenine and thiopurinesuccinic acid at the lowest concentration examined are at least 3 times as effective as a single irradiation with red light. Whether these compounds would be more effective than repeated or continuous exposure to light has not yet been determined. It is difficult to reconcile these findings with the statement by Miller (*12*) that substitution in the amino group is necessary since adenine itself was inactive in promoting expansion of etiolated bean leaves. The conditions he used appear to be identical with ours, except that a buffer was used in our experiments.

Table 1. Control of leaf-disk expansion by light and growth substances. Growth is expressed as increase in diameter, plus or minus standard error, in 48 hours. Each figure represents an average of six separate experiments.

Compound added (μg per dish)	Light treatments			
	Red	Red-far red	Far red	Dark
None	1.22 ± .03	1.00 ± .04	0.99 ± .05	0.68 ± .00
Cobalt*	2.30	1.53	1.47	1.57
Adenine				
0.25	2.20 ± .02	2.21 ± .06	2.19 ± .07	2.23 ± .04
2.5	2.79 ± .02	2.84 ± .05	2.81 ± .04	2.81 ± .04
25.0	2.31 ± .03	2.31 ± .03	2.29 ± .05	2.30 ± .03
50.0	1.96 ± .04	1.96 ± .04	1.99 ± .02	2.05 ± .08
6-(2-Thiopurine)-succinic acid				
0.25	2.39 ± .09	2.44 ± .05	2.38 ± .05	2.37 ± .06
2.5	3.34 ± .06	3.32 ± .06	3.30 ± .04	3.31 ± .07
25.0	2.99 ± .04	3.01 ± .03	3.01 ± .04	3.03 ± .05
50.0	1.84 ± .02	1.86 ± .03	1.84 ± .02	1.91 ± .04
Gibberellic acid				
0.25	2.74 ± .04	1.41 ± .04	1.53 ± .05	1.79 ± .04
2.5	2.56 ± .08	1.53 ± .04	1.41 ± .06	2.01 ± .07
25.0	2.51 ± .06	2.11 ± .07	2.04 ± .03	2.21 ± .06
50.0	2.18 ± .05	1.43 ± .05	1.40 ± .04	2.09 ± .06

* Cobaltous nitrate, $0.98 \times 10^{-4} M$.

As shown in Table 1, the gibberellins also actively promote leaf expansion, both in darkness and in red light, with the red-light effect being at least partially additive to the gibberellin effect. Perhaps the most interesting observation made with the gibberellins is in their interaction with far red light. Thus, far red irradiation not only reverses the red-light effect, but also largely eliminates the effect of the gibberellins at all concentrations tested. The reason for failure of the far red light to cause a significant repression of growth at the 25-μg concentration is not readily apparent. The data presented here also show that the promotion by gibberellin is only slightly affected by concentration and suggest that the interaction with light may be very complex. In any case, it appears that the gibberellins or some complex which they form to promote expansion are very sensitive to far red light. Further experiments are in progress to elucidate this phenomenon.

The results reported for the gibberellins are not in agreement with the statement made by Lockhart (*8*) that gibberellic acid alone is not able to promote leaf expansion of dark-grown peas. Lockhart cited no specific data, but this disagreement in findings may indicate a fundamental difference between the leaf growth systems in beans and peas. On the basis of evidence now available, it is not possible to tell whether the role of gibberellins in promoting leaf growth of beans is the same as its role in reversing the red-light inhibition of elongation growth. These results point up the importance of further and more detailed study of systems exhibiting such growth responses.

Data from other experiments just completed show that adenine and indoleacetic acid interact with light to control leaf expansion (*6*). It will be a very interesting turn of events if it happens that the gibberellins also interact with adenine and indoleacetic acid to control leaf expansion, for a thorough understanding of the mechanism of their interaction would lead to a far better understanding of plant growth processes in general.

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9 April 1957

Anterior Pituitary Preparation with Tropic Activity for Sebaceous, Preputial, and Harderian Glands

In this report, the methods of preparation and assay of a crude extract of anterior pituitary glands of hogs showing tropic effects on sebaceous, preputial, and Harderian glands in the rat are described. These effects can be called sebotropic because the ectodermal glands affected produce secretions rich in lipids.

Lasher, Lorincz, and Rothman (*1*) observed that, in mature, ovariectomized, white rats, hypophysectomy results in atrophy of cutaneous sebaceous glands and loss or greatly diminished responsiveness of these glands to the growth-stimulating effect of progesterone or testosterone. In such rats, furthermore, preparations of corticotropin, somatotropin, follicle-stimulating hormone, prolactin, pituitrin, pitressin, and chorionic gonadotropin all failed to restore responsiveness of sebaceous glands to stimulation by progesterone.

That some pituitary tropic principle is involved in the maintenance of growth responses of the preputial glands (*2*) and Harderian glands (*3*) has also been recognized. In the case of the preputial glands, this pituitary factor was recently claimed to be growth hormone (*4*), whereas, in the case of the Harderian glands, the less definite suggestion was made that growth hormone and possibly even thyrotropin were involved (*5*).

Fresh, anterior pituitary glands of hogs were dried with acetone and extracted with acetic acid by a method commonly used in the preparation of corticotropin (*6*). The remaining residue, from which corticotropin, thyrotropin, and somatotropin had been largely removed, was suspended in water, brought to *p*H 8.6 with dilute ammonium hydroxide, and filtered. The filtrate obtained was clarified with Dicalite, adjusted with dilute hydrochloric acid to *p*H 3.0, and cooled for 2 days at 8°C. The precipitate which formed was then collected, diluted with 0.5-percent phenol solution, and its *p*H adjusted to 6.8. Each milliliter of the resulting preparation (*7*) contained about 4.5 mg of nitrogen. Analysis of 0.2-ml aliquots of the final preparation for glucosamine by a method which could detect as little as 5 μg of the substance (*8*) failed to reveal its presence.

The results of experiments carried out with animals are summarized in Table 1. Hypophysectomized, castrated, male, Sprague-Dawley rats weighing between 100 and 150 g were used following postoperative recovery periods of 5 to 7 days. These animals were periodically weighed, maintained under uniform conditions, and fed *ad libitum* a diet consisting of rat food pellets, horse meat, whole-wheat bread, oranges, carrots, and canned milk. Tap water and saline were available for drinking. Hormones in the dosages indicated in Table 1 were injected subcutaneously into the lower abdominal region each day for 14 days. At the end of this period, the average cutaneous sebaceous gland volume in some rats was determined by the method of Haskin, Lasher, and Rothman (*9*), and the pairs of preputial and Harderian glands from each animal were dissected out and weighed.

It can be seen from Table 1 that sebotropic effects are not associated with the administration of either growth hormone or thyrotropin. It is also noteworthy that the response of Harderian glands to the sebotropic preparation does not require the simultaneous presence of a steroid hormone such as progesterone, as is the case with the responses of preputial (*10*) and cutaneous sebaceous glands.

The sebotropic preparation used in our experiments requires more precise characterization. There is a remote possibility that the observed effects on the growth responses of preputial, Harderian, and cutaneous sebaceous glands might have resulted from some unusual combined action of known pituitary hormones which could have been present in small amounts in our crude extract. There is also the possibility that this sebotropic extract might further be resolved into more specific components. Studies in these directions are in progress.

Besides affecting glands of the sebaceous type, our extract also appeared to influence hair follicle and surface epidermal growth and thickness. These effects, however, are based only on gross impressions, and detailed quantitation is still required.

Table 1. Effects of sebotropic preparation, somatotropin, and thyrotropin individually and in combination with progesterone on glands of sebaceous type in castrated, hypophysectomized rats. Injections were given for 14 days. Data are presented as means ± standard errors. The figures in parentheses in column 2 indicate the numbers of rats from each group which were used in the determination of data on cutaneous sebaceous glands.

Treatment	No. of Rats	Body wt. (% increase)	Preputial glands (wt. in mg/100 g body wt.)	Harderian glands (wt. in mg/100 g body wt.)	Cutaneous sebaceous glands ($mm^3 \times 10^{-5}$/100 g body wt.)
Control	21 (5)	3.0 ± 1.1	11.3 ± 0.6	82.5 ± 2.8	7.4 ± 0.8
Progesterone*	23 (8)	3.5 ± 0.9	12.2 ± 0.8	75.7 ± 2.0	4.8 ± 0.3
Sebotropic preparation†	10 (8)	8.1 ± 2.1	17.0 ± 1.7	101.5 ± 6.1	7.6 ± 1.3
Sebotropic preparation † plus progesterone*	11 (7)	3.1 ± 0.9	42.9 ± 3.1	108.8 ± 5.1	18.6 ± 3.5
Somatotropin‡	14 (6)	24.9 ± 1.6	13.3 ± 0.9	73.8 ± 2.3	6.9 ± 1.7
Somatotropin‡ plus progesterone*	21 (6)	27.1 ± 1.4	15.4 ± 1.4	75.2 ± 2.5	7.7 ± 0.5
Thyrotropin§	5	3.0 ± 1.1	11.0 ± 1.1	77.2 ± 2.6	
Thyrotropin§ plus progesterone*	18	5.8 ± 1.0	12.0 ± 1.0	82.9 ± 2.0	

* Progesterone, 1.0 mg daily (Vitarine Co. No. 4520 and Syntex Co. No. 195). † Sebotropic preparation, 0.25 ml daily. ‡ Somatotropin, 0.15 mg daily (Armour Lot Nos. R491132 and D728076). § Thyrotropin, 1 U.S.P. unit daily (Armour Lot No. M2105).

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