Metamorphosis-Activating System of the Frog

Thyroid hormone feedback matures the neurosecretory mechanism, coordinates the phases of metamorphosis.

William Etkin

Ever since the demonstration by Gudernatsch in 1914 of the effectiveness of thyroid feeding in precipitating amphibian metamorphosis, this remarkable biological phenomenon has furnished material for many of the classical studies in endocrinology. The fundamental studies of B. Allen, Hoskins and Hoskins, P. E. Smith, and E. Uhlenhuth in the two decades following the observation of Gudernatsch established the present concept of the basic endocrine mechanism (*1*). The pituitary secrets a thyroid-stimulating hormone (TSH) which induces the thyroid gland to secrete its hormones (chiefly thyroxin). These act upon the tissues to induce metamorphic change. Of course, innumerable problems of detail in this relationship remained, but by this early work the general analysis had been pushed back to the question of what activates the pituitary in its production of thyroid-stimulating hormone.

Control of Pituitary

In 1934 I undertook to determine whether the pituitary was autonomous in the development of its function or was dependent upon the brain or some other structure for activation. To this end the pituitary primordium was transplanted to various sites in the tail-bud embryo. Though many of the hosts of successful grafts showed no metamorphosis, some did display early metamorphic change. These, however, began transformation late and proceeded at a slower pace than normal. In only one instance was a close approach to the normal rate of early metamorphosis achieved.

Working on a Japanese toad, Uyematsu in 1940 reported the successful removal of the primordium of the posterior hypothalamus, the region of the brain to which the pituitary is normally attached. Some of his animals likewise underwent partial metamorphosis (*2*).

These results indicate that the pituitary is capable of some TSH production when isolated from the brain. I was inclined, as was Uyematsu, to ascribe the failure of complete TSH production to limitations imposed by the experimental situation rather than to lack of association with the brain. Yet this concept of the autonomous development of TSH function in the pituitary was not entirely satisfactory. It offered no explanation of the precise timing of the initiation of metamorphosis and of the correlation of the component elements in the process in the course of normal development. Furthermore, it was not consistent with the picture of reciprocal interaction between thyroid and pituitary that was then emerging from studies in mammals—in particular, with the negative feedback of thyroxin upon pituitary TSH activity, discussed later. However, at that time no new method of approach to the problem seemed available.

The key to the resolution of this difficulty has come from an entirely unexpected direction—namely, from nerve cell cytology. Since 1928 the Scharrers have published a number of reports describing the presence of secretory neurones in the central nervous systems of numerous invertebrates and vertebrates (*3*). In the late 1940's a number of investigators—Bargmann, Hild, Scharrer, and others (*3*)—showed that the pars nervosa of the pituitary is a storage organ for neurosecretion produced in the neurosecretory cells of certain hypothalamic nuclei. Shortly thereafter, Harris, Green, and others (*4*) firmly established the concept that the median eminence of the hypothalamus is an organ through which neurosecretory material is transferred to a pituitary-portal blood system. This portal system conducts the secretory material directly to the anterior lobe of the pituitary, where it influences the release of various hormones, including thyroid stimulating hormone.

The concept which is emerging from contemporary work is that stimuli impinging upon the organism from without and those arising from activities within the organism converge through central nervous pathways upon the hypothalamus. There they influence the neurosecretory neurones and, through these, direct the activity of the pituitary. This in turn directs the function of much of the rest of the endocrine system (*5*).

With the development of these concepts the stage was set for the resolution of the impasse to which our earlier work had led in regard to the regulation of the metamorphic process in amphibians. It is the purpose of this article to develop in outline a coherent theory of the mechanism activating amphibian metamorphosis, based upon recent studies from this and other laboratories, including that of A. A. Voitkevich in the U.S.S.R.

Biphasic Control of Metamorphosis

On the basis of descriptive morphology, the metamorphic process in our common frogs (for example, *Rana pipiens*) may be divided into two phases. The first of these, prometamorphosis, is characterized chiefly by rapid hind-leg growth and lasts about 3 weeks at 23°C. When the hind legs have reached a length slightly greater than that of the body the forelimbs emerge and the period of metamorphic climax sets in. This is characterized by rapid and profound changes in most of the systems of the body. In the course of about 5 days the mouth transforms from the narrow opening of the tadpole to the wide mouth of the frog. The intestinal tract undergoes a com-

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plete reorganization, and the tail and gills are resorbed.

Early studies of the development of the thyroid gland gave evidence of little thyroid activity before metamorphosis, increasing activity of the gland during prometamorphosis, and an extreme degree of activity at climax. Studies of iodine-131 uptake point in the same direction (*6*). We have recently sought evidence on this point by artificially inducing metamorphosis in young tadpoles with different concentrations of externally applied thyroxin (Na-*l*-thyroxin). By this means we found that to match the rate of change characteristic of climax required about 200 parts of thyroxin per billion parts of water; late prometamorphosis required 10 to 20 parts of thyroxin; early prometamorphosis, 3 to 5 parts; and premetamorphosis, less than 1 part. In short, all of these methods indicate a very low rate of thyroid activity before metamorphosis, a rapid buildup of thyroid activity during prometamorphosis, and a sudden flooding of the tissues with thyroid hormone at climax. After metamorphosis the morphology of the gland indicates a return to a very low rate of activity. Since the thyroid activity is controlled by the level of TSH activity of the pituitary, we are led to infer a parallel pattern of activity in this organ. Our fundamental problem thus becomes: How is this pattern so precisely regulated in the normal gland but not in one detached from the brain?

In 1956 I undertook to reinvestigate this problem in terms of the new concepts of brain-pituitary relations. My earlier results with the transplantation of primordia were confirmed, but evidence was secured that even the most successful grafts could produce only prometamorphic changes, including foreleg emergence and initiation of mouth widening, but could not induce tail or gill resorption at appreciable rates. Transplantation of differentiated pituitaries taken from tadpoles in various stages of development into hypophysectomized hosts gave essentially the same results. Metamorphic stasis set in at the beginning of climax despite the persistence of healthy glands active in pigmentary and growth functions. Uyematsu had also earlier reported failure of climax in his hypothalamectomized animals, but since he believed his isolated pituitaries to have degenerated at that time, the significance of this result was overlooked.

My associates and I were able to repeat his experiment in *R. pipiens* and found that the animals lacking the posterior hypothalamus (that is, the infundibulum) were indeed unable to continue into climax. However, in our experimental material the gland was definitely viable. It thus became clear that the isolated pituitary is capable of developing TSH function to the level necessary for prometamorphic transformation, though even here it is erratic and variable. To achieve the high level of function necessary for the induction of climax activity, however, the pituitary requires some influence normally derived from its association with the brain.

The metamorphosis of the typical salamander corresponds to the climax phase of frog transformation with respect to the intensity and kind of metamorphic change and with respect to the extremely high level of thyroid activity occurring at that time. Since the hypothalamic region in the common spotted salamander is much more accessible, in surgical procedures, than that of frog tadpoles, we undertook to test in this species our concept of the dependence of high-level TSH activity upon the connection with the brain. We found that when the connection between the brain and the pituitary in full-grown larvae was cut and the regeneration of tissues was prevented by a collodion barrier, the animals failed to metamorphose (*7*). However, if even a few blood vessels traversed the gap between these organs, metamorphosis ensued. This confirmed the concept of dependence of high-level thyroid activity upon an intact brain-pituitary connection and indicated that the influence of the brain was transmitted through the portal vessels to the pituitary, as had been expected from current neurosecretion theory.

Meanwhile a series of analogous experiments was leading Voitkevich to essentially the same conclusions (*8*). By destroying various parts of the forebrain in tadpoles he showed that lesions that included the hypothalamic level had the specific effect of inhibiting gut and tail reduction (climax events) in tadpoles. He also found that reimplanting the hypothalamic region containing the neurosecretory nuclei restored the capacity for these metamorphic changes. Studies of the normal development of the neurosecretory mechanism suggested increased activity before climax.

The evidence just discussed leads to the concept that the control of pituitary function is biphasic. In the first phase, pre- and prometamorphosis, the pituitary is essentially autonomous in TSH function, although accelerated in its development by its connection with the brain. In the second phase of high activity at climax it is entirely dependent upon the brain and is regulated by way of the neurosecretory mechanism. As we shall see, these two phases are not fundamentally distinct but are parts of a continuous process of pituitary activation under neurosecretory control. However, the level of pituitary function in the first phase is so low that it may occasionally be achieved in the absence of direct connection to the brain. A possible mechanism for this is suggested later.

Correlation of Phases

This biphasic concept of the metamorphic mechanism does not, however, enable us to explain the regularity with which the normal tadpole begins metamorphosis at its species-specific size and how the second—the brain-dependent—phase is correlated with the completion of prometamorphosis. A lead toward the resolution of this problem in terms of the concept developed above came from studies of the growth-promoting influence of pituitary grafts. It was found that a tadpole with its pituitary primordium transplanted to its tail grew more rapidly than normal (*9*). This was interpreted as indicating that the hypothalamus exerts a restraining influence on the production of a growth factor by the normal pituitary. The cytology of the grafts showed that almost all the graft cells had differentiated to a type of acidophil. This type of cell is found in the normal gland, concentrated in the region most remote from contact with the hypothalamus (*8, 10*). We are thus led to believe that neurosecretion from the hypothalamus reaches the pituitary even in the tadpole stage when it acts to inhibit the production of a growth factor by acidophils. We have since obtained evidence that the growth factor is probably prolactin, which is also known to be secreted freely by the isolated mammalian pituitary (*11*).

What is true for the growth factor may also apply to thyroid-stimulating hormone. To consider this we must return to experiments with thyroxin. Studies with goitrogens, first made in

1944 (*1*), have shown that in the tadpole, as in other vertebrates, thyroxin acts, through negative feedback, upon the pituitary to inhibit the production of thyroid-stimulating hormone. Contemporary evidence in work on rats supports the concept that this action is exerted both at the hypothalamic and at the pituitary levels. According to this concept, the level of thyroid hormone in the body is maintained constant by the sensitivity of the system to thyroid feedback, since whenever the level rises too high, negative feedback leads to a reduction in TSH output, and whenever it drops too low the removal of feedback frees the system for great TSH production. Since, as we have seen, the isolated pituitary in the tadpole can, in some instances at least, maintain normal thyroid levels through prometamorphosis, it is clear that the feedback mechanisms here must operate directly upon the TSH cells of the pituitary rather than through the hypothalamus. The low level of thyroxin in the tadpole before metamorphosis must therefore be ascribed to the high sensitivity of the TSH cells of the tadpole pituitary to inhibition by thyroxin at this time. Prometamorphosis must then be accompanied by a progressive reduction in this sensitivity as metamorphosis proceeds, and by a sudden and considerable reduction or even elimination of inhibition at the beginning of climax. Since, as we have seen, TSH production at climax is clearly brain-dependent (that is, neurosecretion-dependent), it appears likely that neurosecretion is the factor acting to desensitize the pituitary cells to thyroid feedback at all stages. On this hypothesis our final problem becomes, specifically how is the pattern of desensitization of the TSH cells of the pituitary achieved by neurosecretion in tadpole development?

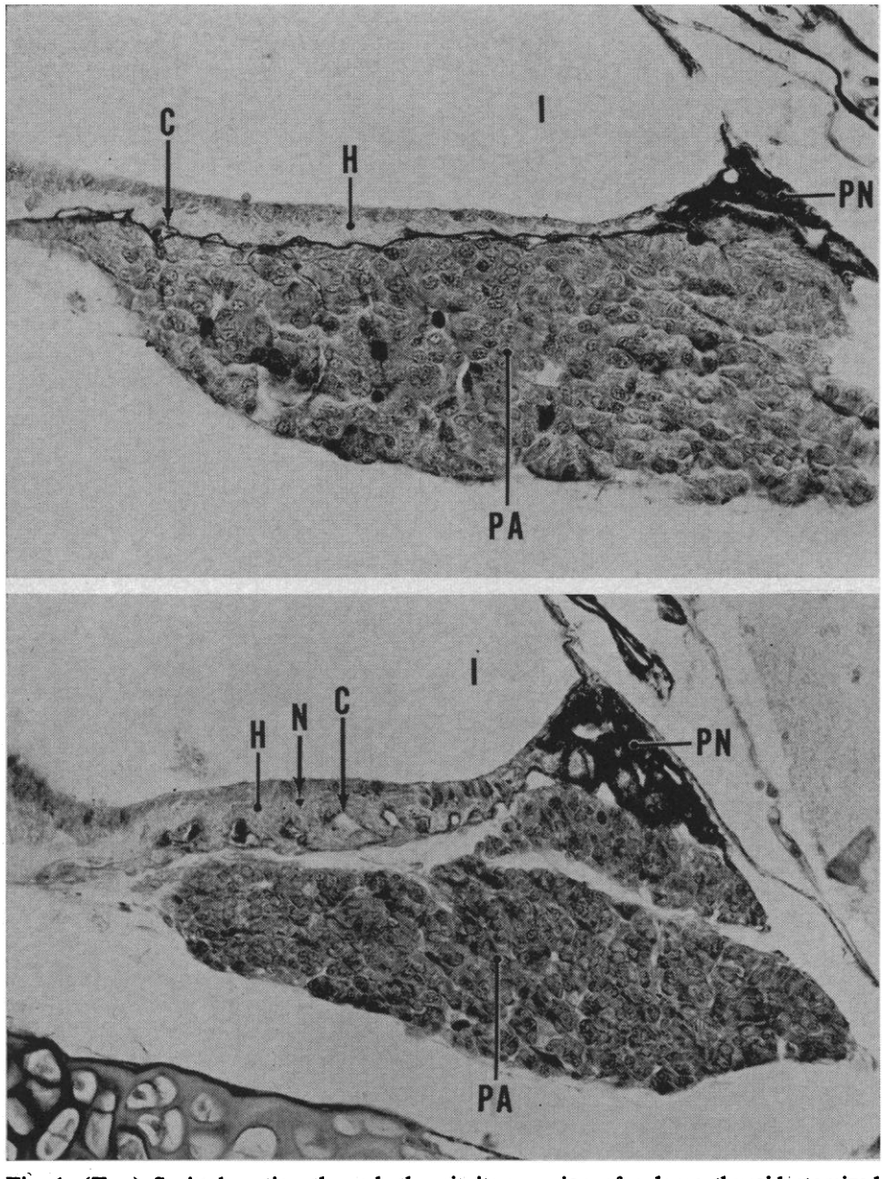

Fig. 1. (Top) Sagittal section through the pituitary region of a large thyroidectomized tadpole which did not metamorphose. (Bottom) Sagittal section through the pituitary region of a similar specimen in which metamorphosis was artificially produced through application of graded concentrations of exogenous thyroxin. Note the thickening of the hypothalamic floor (*H*), the sinking-in of the capillaries (*C*), and the appearance of granules of neurosecretion (*N*) after thyroxin treatment. These are characteristics of the normally differentiated median eminence. *I*, infundibular recess; *PA*, pars anterior of the pituitary; *PN*, pars nervosa.

As I have said, the median eminence is the structure in the floor of the hypothalamus serving to transfer neurosecretory material to the pituitary through the portal veins. In the premetamorphic tadpole, however, the median eminence is not differentiated. Instead there is a diffuse capillary net between the floor of the hypothalamus and the anterior lobe of the pituitary. This network is believed to act in a capacity similar to that of a median eminence, though presumably less effectively. A specialized median eminence develops during prometamorphosis from part of this diffuse system (*8*).

We have found that the thyroidectomized tadpole maintains the undifferentiated larval condition indefinitely. However, if such an animal is treated with appropriate concentrations of thyroxin, the median eminence develops as the animal passes through prometamorphosis, so that when the forelegs emerge this structure has undergone considerable maturation, just as it does in normal metamorphosis (Fig. 1). It is thus clear that the median eminence is stimulated in its development by thyroid hormone and keeps pace in development with the hind legs. Whether the neurosecretory cells are also stimulated by thyroxin action could not be determined in our experimental material, since the species of our investigation is not favorable for the study of these cells. It may be recalled, however, that Voitkevich found increased neurosecretory activity in his material just before normal metamorphosis.

A Self-Accelerating System

We are now in a position to offer a concept of the interaction of thyroid, pituitary, and hypothalamus in regulating the development of the tadpole. The pituitary of the young tadpole produces thyroid-stimulating hormone, but because of the high degree of sensitivity of its TSH cells to negative thyroxin feedback, the level of activity of the thyroid-pituitary system is not permitted to rise above an extremely low level (equivalent to 1 part of thyroxin per billion in the medium). Hypothalamic neurosecretory activity tends to reduce this sensitivity, but since the neurosecretory system is poorly differentiated, its effectiveness is initially minimal. Thyroxin stimulates the maturation of this system, or at least of the median eminence part of it, and thus increases its action in desensitizing the TSH cells. Because the thyroxin level is initially so low, the rate of maturation it induces in the hypothalamus is very low and no progress in the metamorphosis of the body tissues is discernible. This is the premetamorphic period.

The positive feedback action of thyroxin upon the hypothalamus, however, constitutes a self-accelerating system that gathers speed as it advances. When the thyroxin level reaches the equivalent of 3 to 5 parts per billion, the response of the legs to thyroxin is clearly noticeable, and we consider that prometamorphosis has begun. The system is now moving more rapidly, and in the course of about 3 weeks of prometamorphosis it builds up to the 10 to 20 parts-per-billion level, at which time the neurosecretory mechanism has matured sufficiently under the influence of thyroxin to flood the pituitary with neurohormone acting on TSH cells, which become thereby increasingly released from thyroxin inhibition. As a result they pour out an increasing quantity of thyroid-stimulating hormone, and this in turn induces the extreme activation of the thyroid that is characteristic and necessary for metamorphic climax.

When the pituitary is isolated from the brain its production of thyroid-stimulating hormone generally remains extremely low but, as already noted, the isolated pituitary sometimes attains a level of TSH production sufficient for slow prometamorphosis. It is not clear to what this increase is to be ascribed. It may be that there is a desensitization of the cells with aging or conditions associated with grafting. Another possibility is that low concentrations of neurosecretory hormone reach the graft through the systemic circulation. This is suggested by the recent work of Brodish and Long (*12*), who found clear evidence of the presence of a hypothalamic factor in the peripheral circulation of hypophysectomized rats.

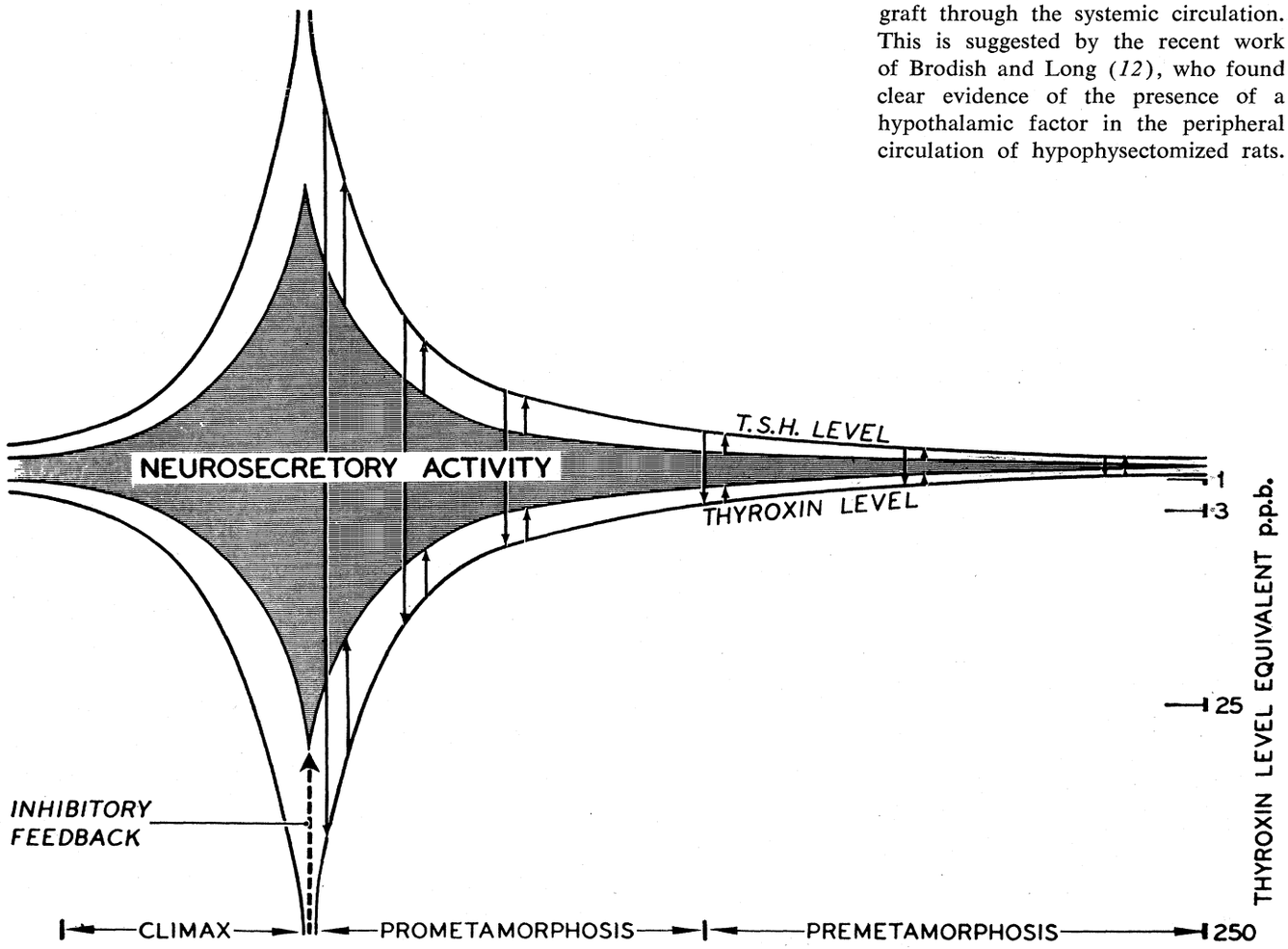

Fig. 2. Schema of interaction of hypothalamic neurosecretory activity, thyrotropin activity, and thyroxin level in the development of the tadpole. Because of positive feedback the system is a self-accelerating one; thus there is an explosive release of thyroxin at the beginning of climax. The solid arrows indicate stimulatory effects (positive feedback); the heavy broken arrow indicates inhibition of the neurosecretory system by the high level of thyroid activity at climax (negative feedback). The direct negative feedback of thyroxin upon the TSH cells of the pituitary is not illustrated.

Other work on the adult frog (*13*) indicated the release of neurosecretory products to the cerebrospinal fluid when the normal pathway is interrupted. In any case, it is clear that the high level of thyroid activity at metamorphic climax depends upon the integrity of the median eminence-pituitary pathway. Since this pathway is itself brought into being by the activity of the thyroid gland during prometamorphosis, climax automatically follows the completion of that process in normal development.

The extreme activation of the metamorphic system at climax completes the maturation of the neurosecretory system. This system, like such other tissues as the legs and tongue, then loses its developmental response to thyroid hormone. The positive feedback effect of thyroid hormone upon the development of the neurosecretory mechanism therefore ceases and is replaced by the negative (inhibitory) effect of high thyroid level upon this system that is characteristic of the adult mammal. According to our present concept, this inhibition removes the substance responsible for desensitizing the TSH cells. With the restoration of their sensitivity to negative thyroxin feedback their activity ceases until the level of thyroxin and thyroid-stimulating hormone falls again to extremely low levels. Hence the thyroid gland becomes inactive, as it is characteristically found to be at the end of metamorphosis. This concept of the metamorphosis-activating mechanism of the tadpole is schematically summarized in Fig. 2.

The significance of the findings reported here may be limited to the amphibians. It is possible, however, that the dramatic events of amphibian metamorphosis are merely extreme examples of processes which take place in all vertebrates, albeit in a more gradual and inconspicuous manner in nonamphibians. In any case, the fact that the differentiation of the median eminence can be suppressed by withdrawal of thyroid hormone and that its normal structure can be reconstituted by treatment with thyroid furnishes an insight into the developmental mechanics of one of the crucial links in neuroendocrine pathways of vertebrates—namely, the median eminence.

References and Notes

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News and Comment

AIBS: Happy Ending in Prospect, But Case Adds to Congressional Skepticism on Support for Science

The American Institute of Biological Sciences (AIBS) appears to be bounding back from its brush with extinction, and, with the crisis now passed, it would be useful to look into some of the broader implications of the institute's misadventures.

First of all, it might be noted that despite a haunting fear that seems to afflict some of the principals in the case, Congress is not very interested in AIBS's woes. Things might change, of course, but at the moment, there is not a flicker of a possibility that any congressional committee will poke into the matter. Persons close to the committees with direct jurisdiction over the National Science Foundation point out that the NSF–AIBS relationship is small pickings when compared with the things they have on their minds early in the session.

These committees include the House Science and Astronautics Committee, which is up to its neck in fending off criticism of the administration's plans to expand the space budget by $2 billion next year, and the Military Operations Subcommittee of the House Government Operations Committee, which finds NASA and the Atomic Energy Commission much more interesting than NSF.

However, while Congress appears to be uninterested in the specifics, the AIBS case—which was well publicized in the Washington press—contributed to the already widespread congressional feeling that something is amiss in relations between science and government, a feeling that the money doled out by Congress for the support of science just isn't being carefully looked after in large segments of the scientific community. The outcome is not going to be reflected in a single decisive act. Rather, it is going to show up in increasing congressional skepticism toward the scientific community. This has already been felt by the administrators of the National Institutes of Health. They are still relatively well off, but the blank check days are going fast, and it is experiences of the AIBS sort that convince Congress that the sooner they go, the better.

Within the scientific community, the AIBS revelations have elicited reactions that suggest that Congress's grounds for concern may not be at all