At the time of the disturbance in the mouse colony, the manufacturer of the pellets had not yet begun regular production of cattle supplements containing stilbestrol. However, it was learned that at least one batch of such feed had been prepared at the request of a local veterinarian and that the same mixing equipment had been used to process both the cattle feed and the mouse pellets. As far as we could determine, the cattle supplement had been processed at about the time of preparation of the first lot of pellets that showed estrogenic activity. The processing equipment presumably contained residual drug for some time afterward, since several subsequent lots of mouse feed also had demonstrable estrogenic activity.

In view of our findings, the use of common processing equipment for the preparation of feeds for laboratory animals and livestock supplements containing stilbestrol would seem to represent a serious potential hazard. All groups concerned—feed manufacturers, breeders of laboratory animals, and the laboratory worker—should be cognizant of the problem, since undoubtedly our experience is not unique.

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Note

1. Roy Hertz, National Cancer Institute, National Institutes of Health, kindly performed the bioassays for estrogenic activity.

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Factors Influencing Curvature in the Avena Test for Plant Hormones

In the present study, attempts have been made to determine factors influencing the response of *Avena* coleoptiles to a given concentration of indole-3-acetic acid (IAA) (*1*). The standard *Avena* test method as described by Went (*2*) was used, but with variations as noted. Standard IAA solutions ranged from 15 to 160 μg/lit.

Preillumination by varying exposures to red light, or complete absence of light, during the 3 days of growth prior to testing did not consistently increase or decrease curvature. Red light is customarily used during this period to prevent elongation of the first internode; this facilitates pulling the primary leaf in the second decapitation but produces no effect on curvature.

Table 1. Effect of time interval between the second decapitation and the application of agar blocks. Each figure for curvature represents the average of six plants in response to the indicated IAA level

Time lapse (min)	Curvature (deg) at IAA levels			
	20 μg/lit	40 μg/lit	80 μg/lit	160 μg/lit
5	21.6	33.1	45.5	43.0
15	16.9	27.6	39.0	37.0
30	18.0	24.0	30.3	27.1
60	16.1	19.0	26.1	25.0

High temperatures, often thought to decrease the *Avena* response, had no noticeable effect. Even at 31 to 32°C, excellent curvatures were obtained.

Subsequently, a marked increase in curvature was found when the interval between the second decapitation and application of agar blocks was held to a minimum. The effect of this time lag is shown in Table 1. This effect was demonstrated repeatedly and without fail. The previous practice in this laboratory had been to decapitate the entire series of test seedlings, that is, 10 rows of 12 plants each, and then to apply the agar blocks, imposing a ½- to 1-hour delay between the operations. The present practice is to apply the agar blocks after each row is decapitated. By this method, a single plant may have a curvature of more than 50 deg in response to 100 μg/lit of IAA. By the previous method, about 30 deg was considered the maximum response.

The time lapse between the first and second decapitation had a minor effect on the curvature response. Table 2 indicates that the longer interval between decapitations is preferable.

Reexamination of data presented by Goodwin (*3*) using the soil culture *Avena* technique and by Schneider and Went (*4*) in recommending a second decapitation points to increased sensitivity in agreement with these findings.

The results of the present investigation suggest that auxin regeneration within the coleoptile tip may be responsible for the striking differences in curvature response. The longer interval between the first and second decapitation would diminish auxin regeneration after the second decapitation. The immediate application of agar blocks would permit the maximum effect of unilateral application of IAA before auxin is uniformly regenerated throughout the coleoptile tip.

Table 2. Effect of time interval between the first and second decapitation. Each figure for curvature represents the average of 8 to 12 plants. Agar blocks applied 10 to 15 minutes after second decapitation, that is, every two rows

Time lapse (hr)	Curvature (deg) at IAA levels			
	15 μg/lit	25 μg/lit	50 μg/lit	100 μg/lit
1	11.4	18.1	26.3	36.4
3*	16.4	22.6	31.9	44.7
4	15.9	29.8	38.3	46.0

* Standard interval.

Supporting evidence is found in the high curvatures reported by Skoog (*5*) in the deseeded *Avena* method in which removal of the seed prevents regeneration.

If rapid regeneration of auxin is the case, it might in turn be influenced by preillumination or high temperatures, making the immediate application of agar blocks more or less critical.

Recent reports in the literature indicate that investigators often rely on small differences between average curvatures of less than 10 deg. Increasing the sensitivity of the *Avena* plants by the immediate application of agar blocks will increase the reliability of results as well as eliminate some of the variability found between laboratories.

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References and Notes

1. Published with the approval of the director as technical paper No. 235 of the Pineapple Research Institute of Hawaii.
2. F. W. Went and K. V. Thimann, *Phytohormones* (Macmillan, New York, 1937), p. 27.
3. R. H. Goodwin, *Am. J. Botany* **26**, 74 (1939).
4. C. L. Schneider and F. W. Went, *Botan. Gaz.* **99**, 470 (1938).
5. F. Skoog, *J. Gen Physiol.* **20**, 311 (1937).

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Influence of Thyroxin and Thyroglobulin on Rice Moth Larva

The influence of thyroxin and other thyroidal preparations in different insects has been studied from time to time by various workers (*1*); and, as has been assumed by Goldsmith (*2*), much of the experimental work carried out so far in this field is open to criticism in that dosages were not adequately controlled (possible improvement of the ration by the thyroid supplement or possible toxicity of higher concentrations), and the insects were not of known ancestry. Further, there was no uniformity in the thyroidal preparations used; many used thyroid extracts, some used hydrolyzed thyroids, while a few others used thyroid substance itself from various mammals. It was therefore considered worth while to reinvestigate this subject by using thyroidal preparations of known potency and the larva of an insect that can be easily grown and a pure strain maintained under standard laboratory conditions.