tupaioid, which he thought demonstrated significant lemuroid characteristics. Since that time *Anagale* has occupied a prominent place in the arguments supporting a primate status for the tree shrews. Simpson (*10*), on the basis of the lemuroid tendencies of *Anagale* and the work of LeGros Clark, classified the tree shrews as the superfamily Tupaioidea and placed them with the lemuriform lemurs in the same infraorder.

This classification has been accepted by many comparative anatomists and by most primatologists and physical anthropologists. Some workers who have dissented include: Evans (*11*), Roux (*12*), Haines (*13*), Straus (*14*), and Osman Hill (*15*). In addition, McKenna (*16*) has recently shown that *Anagale* is neither a primate nor a tupaioid. Usually, the numerous nonprimate characteristics of the tree shrews have been dismissed by attributing them to their primitive insectivore ancestry. In other words they are "characters of common inheritance." This does not appear to be a likely explanation so far as the localization of the pyramidal tract is concerned. In the insectivores examined thus far —the European hedgehog and two species of mole—uncrossed ventral tracts have been reported (*17*). Thus, crossed dorsal pyramidal tracts do not appear to be an insectivore characteristic. Straus (*14*) has suggested that the tree shrews should more properly be placed in a separate order, and our experiments may be interpreted as supporting that conclusion. The present finding does not resolve the controversy, but when considered with other morphological evidence (*11*—*13*) it is suggestive that the tree shrews indeed may not be primates.

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3 December 1964

Gibberellin Production in Pea Seeds Developing in Excised Pods: Effect of Growth Retardant AMO-1618

Abstract. *The gibberellin content of pea seeds developing in excised pea pods cultured on a medium containing the plant growth retardant AMO-1618 was reduced in comparison with pea seeds cultured on retardant-free medium. The reduction increased with increasing concentrations of AMO-1618. However, at the lowest concentration tested (5 milligrams per liter) the growth of the seeds was not affected whereas their gibberellin content was significantly reduced. In conjunction with earlier work on the effect of growth retardants on gibberellin biosynthesis in the fungus* Fusarium moniliforme, *these results indicate that AMO-1618 inhibits the biosynthesis of gibberellins in tissues of higher plants in much the same manner it does in* Fusarium.

The plant growth retardants are a group of chemically unrelated compounds which have the property of reducing the growth of plants. They affect mainly the growth of the stem (internodes); other growth processes and the over-all growth pattern of the plant remain essentially unaffected. The effect of the retardants on stem growth can in most cases be overcome by the application of gibberellin (see *1*).

In previous communications it has been shown that the retardants AMO-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl - 1 - piperidinecarboxylate methyl chloride) and CCC ([2-chloroethyl]-trimethylammonium chloride)suppress gibberellin production in cultures of the fungus *Fusarium moniliforme* (*Gibberella fujikuroi*), the first organism in which gibberellin was discovered, without affecting its growth (*2*). This suppression was found to be due to the inhibition of gibberellin biosynthesis, and not to destruction or inactivation of gibberellin already produced, or other causes (*3*). There is excellent agreement between the growth-retarding effect of a series of CCC analogs in higher plants and their activity in inhibiting gibberellin biosynthesis in *Fusarium* (*4*).

In this report we provide direct evidence that the production of gibberellin in higher plants is inhibited by AMO-1618; this inhibition is very probably the physiological basis for the growth effects of this and, presumably, certain other plant growth retardants.

The experiments were conducted with young fruits (pods) of peas (*Pisum sativum* L., cv. Progress No. 9, a dwarf variety) detached from the plant and grown on a synthetic nutrient medium. The choice of this system was based on several considerations. First, pea seeds, like the seeds of a number of other plants, during certain stages of their development accumulate much greater amounts of gibberellin than are found in seedlings or in the organs of older plants. Our determinations were made mostly on samples of ten seeds; to obtain reasonably reliable results with pea seedlings a minimum of several hundred seedlings are needed per sample. Secondly, in most plants or plant parts the addition of retardants causes a reduction in growth. Even if a reduction in the concentration of endogenous gibberellin is found, the relations between cause and effect remain equivocal. The accumulation of large quantities of gibberellins in developing seeds

offers a means of testing the possibility that the gibberellin content can be reduced without growth being affected. Thirdly, very little is known about the sites of gibberellin biosynthesis in higher plants. Determinations of the gibberellin content of attached parts of a plant may therefore be misleading because the gibberellin may have originated in another part of the plant.

Pods were detached from the plant 13 days after pollination of the flower, were surface-sterilized for 10 minutes with 0.5-percent solution of sodium hypochlorite ("Purex" 9 : 1) and planted with the cut pedicel inserted in a medium consisting of mineral nutrients according to Nitsch (*5*), 5 percent saccharose, and 1 percent agar; each pod was placed in a 60-ml test tube containing 20 ml of the medium. The medium, after the addition of various concentrations of AMO-1618, was sterilized by filtration. The seeds in the explanted pods were fully differentiated but quite small, the pod having nearly reached its final length but being quite flat in shape. The cultures were kept at a temperature of 23°C, and were given 18 hours of light daily (fluorescent plus incandescent lamps, intensity approximately 9.9 lumen/cm^2 at the surface of the culture tubes). The seeds were cultured in this way for 10 days and then removed from the pods and analyzed for their gibberellin content. The period of 10 days was selected because within this time the seeds reach their final size, at least in terms of fresh weight, and their gibberellin content undergoes a striking increase.

The seeds were frozen, ground in dry ice, and extracted twice with cold methanol. The combined extract was evaporated almost to dryness and the residue subjected to thin-layer chromatography, silica gel G being used as the adsorbent and a mixture of isopropyl ether and acetic acid (95:5) as the solvent (*6*). The chromatograms were divided into 10 equal zones and the adsorbent was extracted with wet ethyl acetate. The extractant was evaporated, and the residue was taken up with water containing 0.05 percent of a wetting agent (Tween 20) and tested by two bioassay procedures, both highly specific for gibberellins. One procedure, according to which pea plants are grown in darkness and dwarfed with AMO-1618, depends on the finding that peas may contain gibberellins which are not active on the same peas in the light but are active in the dark (*7*). The second procedure was the dwarf-*d5*-maize test of Phinney (for details of our procedure, see *7*). The results with the two tests were closely comparable and will not be given separately (*8*). In the present experiments, no effort was made to accomplish maximum separation or identification of the different gibberellins which might have been present in the developing pea seeds. The results reported are total activities per seed, expressed as equivalents of gibberellin A_3 (gibberellic acid).

Table 1. The effect of AMO–1618 on the growth and gibberellin content of pea seeds developing in excised pods. Ten seeds were used for each determination, except before culture—that is, when the pods had just been detached from the parent plant—when 35 seeds were used.

Conc. of AMO-1618 in medium (mg/l)	Fresh weight of seed		Gibberellin content per seed	
	In grams	Percent	Gibberellin* (μg)	Percent
Before culture				
	0.0027		0.0015	
After 10 days of culture with AMO–1613				
	0.31	100	0.430	100
5	0.30	97	0.173	40
50	0.26	84	0.055	13
500	0.07	23	0.04	9

* Results expressed as equivalents of gibberellin A_3, in micrograms.

The results, which are summarized in Table 1, indicated that during the 10-day culture period the weight of the seeds increased by a factor of approximately 100 while the gibberellin content of each seed increased by a factor of approximately 300. The addition of AMO-1618 to the culture medium depressed both the growth and the gibberellin content of the seeds. However, these two effects were not parallel. At the lowest concentration of AMO-1618 (5 mg/lit.), the growth of the seeds was unaffected but the gibberellin content was reduced by 60 percent. At 50 mg of AMO-1618 per liter the reduction in gibberellin content (87 percent) was also still substantially greater than the reduction in growth (16 percent). Only at a concentration of 500 mg of AMO-1618 per liter were both growth and gibberellin content reduced in approximately equal ratios.

The results with AMO-1618 at the lower concentrations are of particular significance. They eliminate the possibility that the changes in the gibberellin content of the seeds are the consequence rather than the cause of the changes in growth. The only remaining factor of uncertainty is that the seeds contain a mechanism for the destruction or inactivation of gibberellin, or materials which interfere with gibberellin action in the bioassays, and that AMO-1618 enhances the activity of these systems. However, there is no evidence for any of these possibilities (*9*). Thus, particularly when considered in conjunction with the results in *Fusarium* (*2–4*), our results are strong evidence that AMO-1618, and very probably also CCC and related retardants, can inhibit gibberellin biosynthesis in higher plants, as they do in the fungus.

As discussed before (*4*), this interpretation does not rule out other effects of growth retardants. In several instances (for example *10*) it has been found that the retardants inhibit the growth of certain plant tissues but that this inhibition is not overcome by the addition of gibberellin. However, in those numerous cases in which the effect of the retardants on growth and developmental responses of plants can be fully reversed by gibberellin, it is very likely that this effect is based on the inhibition of gibberellin biosynthesis which leads to a deficiency of physiologically active gibberellins in the plants. With these findings, the retardants AMO-1618 and CCC are among the few known synthetic plant-growth-regulating chemicals whose major action can be pinpointed to one very definite physiological or biochemical mechanism. Such substances may therefore become very useful tools in studies on the growth physiology of plants. A corollary result is that developing pea seeds are dependent on only a fraction of the gibberellins synthesized at this stage.

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21 October 1964

Genetic Influence on Experimental Allergic Thyroiditis in Guinea Pigs

Abstract. *After immunization with low doses of guinea pig thyroid extract, incidence of experimental allergic thyroiditis is greater in the Hartley strain of guinea pig than in strain 13.*

In response to immunization, some strains of animals produce more antibody than others (*1*). These differences in immune responsiveness, apparently genetic in origin, may also affect the incidence of an experimentally induced autoallergic disease. One of these, experimental allergic encephalomyelitis, develops in mice of the BSVS strain more frequently than in other strains (*2*). Similarly, after immunization with central nervous system tissue emulsified in Freund's complete adjuvant, the Hartley strain of guinea pig develops encephalomyelitis in response to doses of the central nervous system antigen which are insufficient to cause the disease in several other strains (*3*). These findings suggested that strain differences might influence the development of another experimental autoimmune disease, allergic thyroiditis. Consequently, two strains of guinea pigs were immunized with selected doses of guinea pig thyroid antigen. The resulting incidence of thyroiditis served as the index of the genetically determined proclivity of each strain to develop this disease.

Random-bred Hartley and inbred strain 13 (*4*) male guinea pigs (*5*) were used for these experiments. They were housed, five to a cage, in one room, similarly cared for, and provided continually with Feed A (*6*) pellets, kale, and carrots. On attaining a weight of 400 to 500 g they were injected with a saline extract of guinea pig thyroid emulsified in Freund's complete adjuvant; the extract was prepared as previously described (*7*) and diluted with saline to the desired concentration, and three volumes were emulsified with one volume of complete adjuvant comprising 80 percent Bayol F, 20 percent Arlacel A, and 4 mg per milliliter of *Mycobacterium tuberculosis* H37Rv. One milliliter of the emulsion was injected intradermally in small portions at approximately 20 sites distributed over the hind feet, legs, and rump. The serial twofold dilutions of the stock thyroid extract were prepared at one time and individually emulsified with the adjuvant; each emulsion preparation was injected into five to ten guinea pigs of each strain.

Six weeks after immunization the animals were killed with ether. The thyroid glands were excised and the right and left lobes were separately fixed in neutral buffered 10 percent formalin and embedded in paraffin. Serial sections were cut at 6 μ through the long axis of each lobe until 0.1 to 0.4 mm of the tissue had been sectioned; the sections were stained with hematoxylin and eosin. The presence of thyroiditis was determined by the appearance of inflammatory reaction in the gland, according to previously described criteria (*7*).

The results (Table 1) show in both strains a relation between the dose of thyroid extract and incidence of disease. All animals given doses of greater concentration than the 1:1280 dilution developed disease, irrespective of strain. As the concentration of thyroid extract decreased, disease incidence diminished in both strains, but did so more slowly in the Hartley strain, until a dose insufficient to produce disease in either strain was reached; this was the 1:40,960 dilution.

Statistical comparison of the frequency of thyroiditis in all the Hartley and strain 13 guinea pigs (injected with the dilutions 1:1280 to 1:20,480, inclusive) indicated that the greater incidence in the Hartley guinea pigs was significant at the 1-percent level, ($p < .01$, by the chi-square test). Similar analysis for each dilution showed that the difference in disease incidence at the three dilutions, 1:1280, 1:2560, and 1:5120, was also statistically significant ($p < .01$).

Many experimental and environmental factors may influence the immune response and could thereby affect the incidence of experimental allergic thyroiditis. Variables such as the nature of the antigen, type of antigen-adjuvant emulsion, immunizing dose, sex, weight, and nutritional status of the animals were controlled. Certain other factors in the environment, such as infection or the normal flora (and their effects on the immune mechanism), are more difficult to regulate; we attempted to control these factors by regulating the housing and care of the animals before immunization and throughout the experiments. Thus, the greater reactivity of the Hartley strain compared with that of strain 13 appears to result from a genetic difference.

Table 1. Incidence of thyroiditis in Hartley and strain 13 guinea pigs immunized with various dilutions of thyroid extract in Freund's complete adjuvant.

Dilution of thyroid extract	Guinea pigs*	
	Hartley	Strain 13
1:640	5/5	5/5
1:1280	10/10	4/10
1:2560	9/10	2/10
1:5120	6/10	0/10
1:10,240	2/10	2/10
1:20,480	1/5	0/5
Total †	28/45	8/45

* Animals with disease/total number of animals.
† Excluding the 1 : 640 dilution.

Genetic influence on the frequency of this disease is not confined to the guinea pig; the experiments of Gorstein *et al.* (*8*) show the incidence of allergic thyroiditis in mice to be greater in the Swiss strain than in the black C_{57} strain. The mechanism of the genetically determined response remains to be elucidated. It may reflect the degree and type of the immune response to a given stimulus, or a differing extent of tissue damage secondary to the same degree of immunity.

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