COUMARIN AS THE STIMULATOR OF GROWTH OF ETIOLATED MAIZE STEM TISSUE

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THAT coumarin (*o*-hydroxy-*cis*-cinnamic acid lactone) is a substance with a physiological activity has been known for a long time.[1] At first it has been generally regarded as an inhibitor of seeds germination[2-4] and growth of seedlings, but later some evidence has been gathered that effects induced by this substance depending upon condition, plant genus, kind of tissue, its own concentration, etc.,—might be diverse, and there are well documented findings that coumarin can function as a naturally occurring plant growth regulator.

In 1938 Grace,[5] based on results of his experiments, first concluded that coumarin probably plays a role of an auxin. Some years later Thimann and Bonner,[6] and Gantzer[7] reported that in certain cocentrations coumarin acts synergistically with indole acetic acid (IAA), and finally Neumann,[8] who examined effects of coumarin on the longitudinal elongation of sunflower hypocotyls and similar tissues of other plants, concluded that this substance should be regarded as an auxin,[9] with the mode of action differing from that of IAA and, probably of gibberellin (GA) too.

The growth-promoting action of coumarin is very interesting and, in order to obtain some further evidence of the generality of this phenomenon, we have performed experiments with maize seedlings.

Maize seeds (*Zea mays*, var. Wir. 42), after 3 hours period of soaking in water, were grown in sterilized quartz sand at 26° C. in darkness except for occasional diffused daylight. Five days later, 10 mm. segments were cut from the stem, 5 mm. below the leaf node (the plants in this stage were 5 cm. long) and the sections were floated on distilled water for 2·5 hours, then divided into lots of 15, carefully dried on blotting paper and—after quickly weighing—were floated on surfaces of the test solutions poured into small petri dishes.

In the control sample, instead of coumarin, 10 ml. of distilled water were poured.

After 20 hours period of incubation under diffused fluorescent tube light at room temperature (+ 22·5° C.) the segments were taken out and their increase in weight were measured (preliminary experiments have shown that the increase in weight runs parallel to longitudinal elongation of sections).

From the results presented in Table I it is evident that coumarin significantly accelerates the growth (longitudinal elongation) of sections, and the level of this stimulation depends upon concentration of this substance. In solutions 250 and 500 p.p.m. increase in weight reaches about 255% of control value taken as 100%. The range of concentration of coumarin is optimal—more concentrated solutions produce inhibition and coumarin in 1,000 p.p.m. is toxic for the plant: the longitudinal extension of sections is stopped and at the end of incubation they are flexible, look brownish, and seem to be killed. It is necessary to say that in solution of 500 p.p.m. at the end of 20 hours incubation period the segments are too slightly flexible; additional experiments revealed that the growth rate of sections treated by coumarin is not constant with time—the growth rate reaches its maximum value within the first 4 hours and then it decreases (it was noted that in the first hours of treatment, the growth of sections floated on the surface of 500 p.p.m. coumarin solution was accelerated to about 450% of control, and slight stimulation was observed in 1,000 p.p.m., too).

In the literature there are some evidences supporting the idea that coumarin influences the water relation of plants.[10-12] Therefore, in addition to fresh weight we have also measured the dry weight of samples at the start and at the end of incubation. As in the test solutions there was no sugar or other nutritional compounds—it is clear that the increase in fresh weight of samples must be attributed to absorption of water only, and the dry matter of samples would be reduced as a result of CO_2 output in the respiration. The truth of this *a priori* supposition will be apparent from the following observations (*see* Table I):—In the sample treated with distilled water (control)—in the time of experiment the fresh weight of sections increases from the initial 100 mg. to the final 117 mg., and the percentage of dry matter calculated from the equation:

$$\frac{\text{dry matter}}{\text{fresh weight}} \times 100$$

falls from 5·97 to 5·06; in absolute values the dry matter changes from about 5·97 mg. to 5·93 mg. so that the net loss

TABLE I

Effect of coumarin in different concentrations on the growth of excised maize stem tissue through 20 hours period of incubation

	Initial values	Concentrations of coumarin, in p.p.m.						
		0 Control	10	50	100	250	500	1000
Fresh weight, mg. ..	100·0	117·1	120·5	121·5	128·2	136·7	137·0	117·0
Increase of fresh weight, in % ..	..	100	120	126	165	215	216	96
Dry matter, mg. ..	5·97	5·93	6·14	6·11	6·10	5·86	5·54	5·23
Change of dry matter, mg. ..	..	−0·04	+0·21	+0·18	+0·07	−0·11	−0·43	−0·74
$\frac{\text{Dry matter}}{\text{fresh weight}} \times 100$	5·97	5·06	5·10	5·03	4·76	4·29	4·05	4·44

All values are average of 6–8 repetitions.

of weight equals 0·04 mg. In the same time in solutions 10, 50 and 100 p.p.m. of coumarin dry matter increases by 0·21 mg., 0·18 mg., and 0·07 mg., respectively, and in more concentrated solutions it significantly decreases (*see* Table I).

Because the rise of fresh weight is accompanied by the decrease in dry matter, it might be concluded that stimulation of growth caused by coumarin is caused—partly at least—by acceleration of water absorption by sections. The increase in dry matter in solutions 10-100 p.p.m. is not clear; but the significant decrease of dry matter at 250 and 500 p.p.m. may be the result of simultaneous stimulation of respiratory activity of tested sections induced by coumarin and more rapid dissimilation of sugars on this way (results not yet published)—solution of 1,000 p.p.m. of coumarin probably additionally kills the tissue and this leads to diffusion of cellular components to the surrounding fluid.

In order to obtain some evidence concerning the mechanism of the growth-promoting activity of coumarin described we performed comparative experiments with indole acetic acid and gibberellin. While the details of these will be published elsewhere, here it is necessary only to say that our results are similar to those reported by Neumann[9] for sunflower hypocotyls and it may be concluded that the nature of coumarin action in promoting the growth of excised maize stem sections differs from that of IAA and GA, but it is doubtful whether coumarin may be named an auxin.

1. Klebs, A., *Die Bedingungen der Fortpflanzung bei einigen Algæ und Pflanzen*, Jena, 1896.
2. Sigmund, W., *Biochem Z.*, 1914, **62**, 339.
3. Kuhn, R., Jerckel, D, Moewus, F., Moller, E. F. und Lettre, H., *Naturwiss.*, 1943. **31**, 468.
4. Evenari, M., *Symp. Soc. Exp. Biol.*, 1957, No. 11, 21.
5. Grace, N. H., *Canad. J. Res.*, 1938, **16 C**, 143.
6. Thimann, K. V. and Bonner, W. D., *Proc. U.S. Natl. Acad. Sci.*, 1949, **35**, 272.
7. Gantzer, E., *Planta*, 1960, **55**, 235.
8. Neumann, J., *Science*, 1959, **129**, 1675.
9. —, *Physiol. Plantarum*, 1960, **12**, 328.
10. Guttenberg, H. und Beythien, A., *Planta*, 1952, **40**, 36.
11. Blaim, K., *J. Exp. Botany*, 1960, **11**, 377.
12. Knypl, J. S., *Naturwiss.*, 1960, **47**, 524.

NUCLEI FOR RAIN FORMATION

INTERESTING evidence has been obtained on the possible origin of the minute, mysterious, and so far, unidentified particles whose presence in the atmosphere is vital to the formation of rain from supercooled cloud. This has resulted from a study of samples collected at high altitudes by U-2 aircraft operated by the U.S. Air Force from a base near Sale, Victoria. Thanks to the generous co-operation of the U.S. authorities, special dust-collecting filters were fitted to the U-2 aircraft. Samples of dust present at heights of 50,000–60,000 feet were obtained from ocean areas well to the south of Australia.

The results show unmistakably that substantial numbers of particles occur at these high altitudes—very many more than can be accounted for if they all come from the Earth's surface. Support is thus given to a theory first advanced by Dr. E. G. Bowen in 1953 that an important source of these particles is the meteor dust which the earth picks up, in the course of its annual journey round the Sun, as it intersects the orbits of the various well-known streams of meteors. This meteoritic dust takes some 30 days to sink to the cloud-bearing levels of the atmosphere, and stimulates heavy rainfall when it arrives there if meteorological conditions happen to be favourable.