LEAF GROWTH FACTORS II—THE ACTIVITY OF PURE SUBSTANCES IN LEAF GROWTH

BY DAVID M. BONNER AND A. J. HAAGEN-SMIT

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated March 14, 1939

It was shown by Went[7] that leaf growth in etiolated pea seedlings is primarily controlled by substances supplied from the cotyledons. Vein growth is controlled by auxin,[2] while growth of the mesophyll is independent of auxin, and it is the factors concerned in the growth of the latter which we will discuss in this paper. In a recent paper[3] a bio-assay for these factors was described in detail, as well as a variety of sources, and so will be discussed only briefly here. An outline of the leaf test is as follows: Circular discs ca. 19.5 mm.^{2} in area are cut from young first foliage leaves of *Raphanus*. Twenty such discs are floated on the solutions to be tested, and allowed to grow on these solutions for 30 hours at 25°C. The total wet weight of all 20 sections from a single solution is then determined by direct weighing in a standard manner. All solutions to be tested are made up to contain 1% sucrose, and activities are compared to the growth of sections grown in 1% sucrose alone. The source from which a solution of standard activity (S. S. A.) is made is the pea diffusate described by Kögl and Haagen-Smit.[6] The present paper will discuss only the activity of various pure substances in increasing mesophyll growth.

TABLE 1

ACTIVITY OF AMINO ACIDS IN THE LEAF TEST USING RAPHANUS LEAVES AS TEST OBJECTS. ACTIVITY ON BASIS OF GROWTH IN 1% SUCROSE SOLUTION IS EQUAL TO 100

ACTIVE AMINO ACIDS	CONCENTRATION EXPRESSED AS MG. PER CC. OF 1% SUCROSE SOLUTION			
	0.5	0.1	0.02	0.005
Proline	124	114	107	105
Asparagine	116	112	103	101
d-Valine	112	105	103	100
Glutamic acid	toxic	108	102	100
Alanine	110	100	100	100
Leucine	110	100	100	100

Amino acids tested between the concentrations of 0.5–0.005 mg. per one cc. of 1% sucrose solution and found to be inactive:

Histidine, glycine, aspartic acid, arginine, citrulline, serine, phenyl-alanine, cystine and beta-alanine.

In table 1 are listed the amino acids that have been tested in the leaf test. When radish leaves are used as test objects asparagine and proline proved the most active. However, in the case of the amino acids it seems

likely that the species of plant from which the leaf is taken determines the amino acid requirements; thus when *Nicotiana sylvestris* is used as the test object arginine proved to be the most active, while with *Raphanus* leaves arginine was totally inactive. However, the growth obtained by the use of amino acids is never as great as that obtained for the S. S. A., nor are the amino acids active by themselves at high dilution. Table 2 lists a variety

TABLE 2

SUBSTANCES TESTED BETWEEN THE CONCENTRATIONS OF 0.5–0.005 MG. PER CC. OF 1% SUCROSE SOLUTION, AND FOUND TO BE INACTIVE IN THE LEAF TEST USING *Raphanus* LEAVES AS TEST OBJECTS

Nicotinic acid	Vitamin B_1
Nicotinic acid amide	Vitamin B_2
Indole (3) acetic acid	Vitamin B_6
Theelin I	Vitamin E
Inositol	2-methyl, 6-amino pyrimidine
Biotin	2-methyl, 6-hydroxy pyrimidine
Boric acid	Thiazole of vitamin B_1
	Uracil

of substances known to be active in different biological phenomena which have been tested in the leaf test. In every instance they have been found to be inactive with the exception of nicotinic acid amide which showed slight activity at a rather high concentration. Yeast nucleic acid was found to possess very definite activity. Upon testing of various crystalline purines, adenine proved to be active at the highest dilution, 20 gamma per liter, hypoxanthine had activity though at a somewhat higher concentration, while guanine, xanthine, uric acid and caffeine had slight activity or were totally inactive (table 3).

TABLE 3

ACTIVITIES OF PURINES IN THE LEAF TEST USING *Raphanus* LEAVES AS TEST OBJECTS. ACTIVITY ON BASIS OF GROWTH IN 1% SUCROSE SOLUTION EQUAL TO 100

PURINE TESTED	CONCENTRATION EXPRESSED AS MG. PER CC OF 1% SUCROSE SOLUTION 0.5	0.1	0.05	0.01	0.005
Adenine	...	...	118	117	113
Hypoxanthine	...	109	116	104	100
Xanthine	...	115	107	100	100
Caffeine	102	112	108	100	100
Uric acid	118	108	100	100	100
Guanine	100	100	100	100	100

That adenine, or a related purine, is the only factor concerned in mesophyll growth seems unlikely not only from chemical studies of the pea diffusate to be reported later, but also from the fact that in only one or two instances was the amount of growth obtainable from leaf sections in adenine plus 1% sucrose as great as that obtainable from the S. S. A. This means,

as shown in table 4, that the amount of growth at the optimum concentration of adenine was not as great as that obtainable at the optimum concentration of the S. S. A. Due to this definite activity it was deemed of interest to test its activity in a variety of ways.

The first type of experiment was that of culturing entire etiolated pea seedling leaves. Etiolated pea seedlings were germinated and grown sterilely in test tubes on nutrient agar. Ten days after germination the leaves, which were very small and curled, were cut off and transferred by sterile technique to 50 cc. Erlenmeyer flasks. The basic inorganic medium used was that used in the culture of pea roots,[4] 20 cc. of medium being used per flask. The following series of cultures were used: A—water; B—inorganic medium plus 1% sucrose; C—B plus 2 mg./l. of adenine; D—B plus 0.2 mg./l. of adenine; E—B plus 200 mg. dry weight of pea diffusate per liter. Ten flasks, with three leaves per flask, were used for each series. After five weeks, observations on the growth in the various solutions were

TABLE 4

COMPARISON OF THE ACTIVITIES OF ADENINE AND PEA DIFFUSATE IN THE LEAF TEST USING *Raphanus* LEAVES AS TEST OBJECTS, WITH COMPARISON ON SEVERAL DIFFERENT DAYS

Activity on Basis of Growth in 1% Sucrose Solution Is Equal to 100.

DATE OF TEST	ACTIVITY AT OPTIMUM CONCENTRATION OF PEA DIFFUSATE	ACTIVITY AT OPTIMUM CONCENTRATION OF ADENINE
November 5, 1938	125	118
November 9, 1938	135	125
November 12, 1938	113	100
November 14, 1938	130	110
December 1, 1938	118	107
January 13, 1939	118	108

made. A concentration of 2 mg./l. of adenine proved inhibitory, while the growth of the leaves in the flasks to which adenine had been added at a concentration of 0.2 mg./l. was greater than in the series with only inorganic medium plus sucrose, though in no instance was the growth as great as that in pea diffusate.[3] Thus in this test the behavior of adenine is similar to that in the leaf test.

The effect of adenine upon the growth of isolated pea roots was also investigated.[8] The method of culturing isolated roots has been reported in detail elsewhere,[4] so will not be described here. In the first six transfers the addition of adenine produced no increase in growth above that of roots growing on vitamin B_1 and nicotinic acid.[1] After six transfers, i.e., after six weeks, adenine began to show some beneficial effect, this effect becoming greater with increase in number of transfers. Although one can conclude that adenine may be a growth factor for pea roots under our conditions of

culture, the effect produced is much smaller than that of other factors as vitamin B_1 and nicotinic acid.[1]

The third type of experiment carried out with adenine was a determination of its effect on *Cosmos* plants grown in the greenhouse in washed sand and supplied with nutrient solution under conditions similar to those of Bonner and Greene.[5,8] Shive's nutrient solution was used, with adenine added to it in the concentrations of 0.5 mg./l., and 0.1 mg./l., each pot receiving 250 cc. of nutrient on alternate days. One pot was used for each

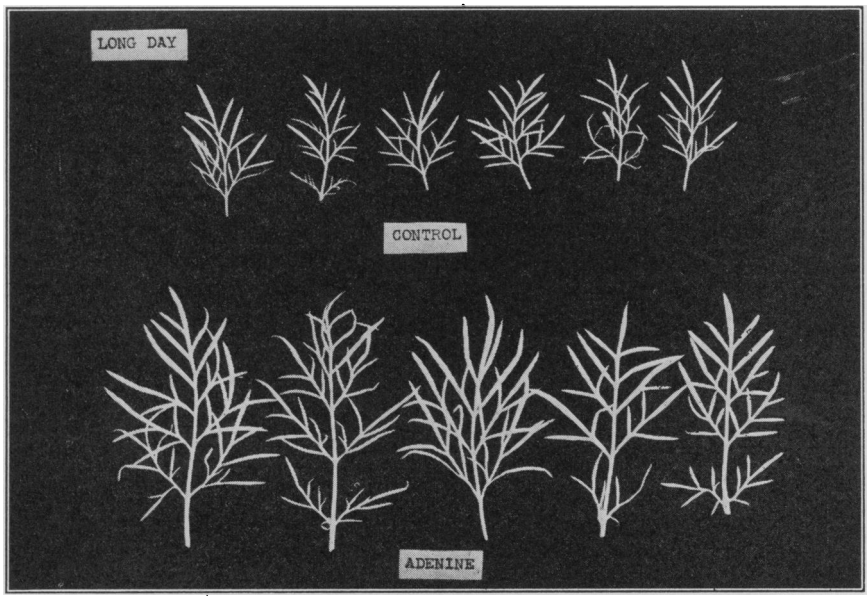

FIGURE 1

Leaves from plants grown with and without adenine.

Control: leaves from *Cosmos* plants grown in washed sand and watered with Shive's nutrient solution, five weeks after germination.

Adenine: leaves from *Cosmos* plants grown under identical conditions but with the addition of 0.1 mg./l. of adenine to the nutrient solution.

of these concentrations, with two pots for controls, and similar series were run on both short and long photoperiods. No appreciable effect appeared during the first two to three weeks after germination. After three weeks the plants treated with 0.1 mg./l. of adenine became steadily larger than the controls with markedly larger leaves. A concentration of 0.5 mg./l. proved in this case to be inhibitory. The effect on size of the plants was much greater for plants on a long photoperiod, with the effect on leaf size being more pronounced in plants growing on a short photoperiod. Figure 1 shows a comparison of leaf size between control plants and treated plants

(0.1 mg./l. of adenine) five weeks after germination. Each leaf was taken from a separate plant, the position of the leaf on the plant being in each case the same. Thus it appears that the addition of adenine to plants can under certain conditions bring about an increase in leaf area.

Discussion.—From a study of the activity of pure substances in the leaf test, one general class of compounds appears to have activity, namely, the purines, with adenine possessing the highest activity of the purines tested. The naturally occurring purine active in leaf growth must await isolation and identification, this work being in progress. It should be emphasized that factors other than adenine, or any single purine, are involved in leaf growth, as was pointed out earlier in this paper. Therefore, it is not surprising that the growth of excised etiolated pea seedling leaves, as has been shown, is not very great. The leaves themselves have but little of these other factors, as shown by the large response to pea diffusate,[3] and the culture conditions are such as to largely preclude their synthesis. They are not furnished with possibly essential amino acids, nor are the light conditions necessarily correct. However, when one gives an excess of one factor to a plant growing normally in a greenhouse, the conditions are considerably different. By increasing the growth rate of the leaves under normal conditions to an extent shown by adenine in the leaf test, the other necessary factors will be formed in greater amount, with the result that the growth rate of the leaves may be further increased. The increase in leaf surface can adequately account for the enhanced growth of the plant as a whole, not only by increasing carbohydrate synthesis but also by increasing phytohormone synthesis. For example, auxin, a stem growth hormone, is known to be produced in green leaves, so that its production may be increased. Vitamin B_1, a root growth hormone, is also known to be synthesized in green leaves. Thus the increase in leaf surface would indirectly cause increased stem and root growth, the resultant plant being normally proportioned, but of larger size and increased general vigor.

[1] Addicott, F. T., and Bonner, James, *Science*, **88**, 577 (1938).

[2] Avery, G. S., Jr., *Bull. Torrey Bot. Club*, **62**, 313 (1935).

[3] Bonner, David M., Haagen-Smit, A. J., and Went, F. W., *Bot. Gaz.* (in press).

[4] Bonner, James, and Addicott, F., *Bot. Gaz.*, **99**, 144 (1937).

[5] Bonner, James, and Greene, J., *Bot. Gaz.*, **100**, 226 (1938).

[6] Kögl, F., and Haagen-Smit, A. J., *Zeit. physiol. Chem.*, **243**, 209 (1936).

[7] Went, F. W., *Plant Physiol.*, **13**, 55 (1938); *Ibid., Am. Jour. Bot.*, **25**, 44 (1938).

[8] The authors are indebted to Dr. James Bonner for these experiments.

[9] The authors wish to thank Mr. L. E. Castle, Jr., for aid in the carrying out of the leaf tests.

[10] Report of work carried out with the aid of the Works Progress Administration. Official Project Number 665-07-3-83, Work Project W-9809.