sulfonate-C^{14} produced one mole of formic acid. The structure of the sulfolipid consistent with the observed properties is 1-O-(β-6′-deoxy-aldohexopyranosyl 6′-sulfonic acid)-3-O-oleoyl glycerol. The sulfonic acid structure suggests its origin by peroxidation of an -S-S- linked glycolipid in the photosynthetic apparatus.

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† Part of the material in this paper is included in the thesis submitted by R. Wiser to the Graduate School of The Pennsylvania State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, August, 1958.

‡Available structural evidence is also consistent with an isomeric 1-deoxyketopyranoside 1-sulfonic acid structure.

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*AN EFFECT OF AUXINS ON THE HEAT COAGULABILITY OF THE PROTEINS OF GROWING PLANT CELLS**

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Most current theories of auxin action are based on the discovery by Heyn[1] that the application of auxin to growing plant cells results in an increased plastic extensibility of the cell wall. While such a mechanism suffices to explain elongation induced by auxin, it is obviously inadequate to account for the known effects of

auxin on the cytoplasm[2-8] and the nucleus,[9] or to explain the mitogenic or morphogenetic aspects of auxin action.

We have recently conducted experiments with C^{14}-carboxyl-labeled 2,4-dichlorophenoxyacetic acid (2,4-D) fed to growing pea stem sections in an attempt to localize the region of the cell in which auxin acts. A centrifugal fractionation of the cell contents after several hours of auxin-induced growth revealed the label not to be attached to any cell organelle, but rather to be localized in the final centrifugal supernatant fraction devoid of all particulates. When we attempted to precipitate the proteins in this supernatant fraction to determine whether any of the labeled auxin was protein bound, we discovered fortuitously that the proteins derived from cells treated with the auxin failed almost completely to coagulate after 10 minutes of boiling, while proteins of the control cells produced a copious white precipitate under the same conditions. Repetition of this experiment with various auxins and analogs, and with quantitative measurement of the precipitate, revealed a close correlation to exist between the effect produced on the protein and the effect produced on growth. We thus consider that this phenomenon, which we interpret as an auxin-induced alteration of the physical state of the cellular proteins, may be important in explaining auxin action.

Materials and Methods.—Alaska peas, obtained from Associated Seed Growers, New Haven, Connecticut, were soaked in tap water for three hours and sown in water-saturated vermiculite in polyethylene containers. They were then grown in darkness for 7 days at a temperature of 27° ± 1°C. At the end of this time, 10 mm-long subapical stem sections of the third internode were excised with a double-bladed cutting tool and used for the overnight incubations. Approximately 80 such sections (the number varied slightly between experiments) were placed in 10 ml of growth medium in a 9-cm petri dish and incubated overnight (*ca.* 18 hr) in the dark room. The incubation medium contained 2 per cent sucrose, 0.02 *M* pH 6.1 phosphate buffer and the auxin or auxin analog, where added. The growth of the sections was estimated by length and fresh weight measurements.[10] After harvest, the sections were rinsed, homogenized in a pre-chilled mortar and pestle with 14 ml of cold 0.5 *M* sucrose + 0.001 *M* ethylenediaminetetraacetic acid (EDTA), strained through four layers of washed cheesecloth, and centrifuged at 24,000 × *g* for one hour in a water-cooled Sorvall centrifuge in the cold room. The final volumes of the decanted supernatant liquids were then equalized with fresh sucrose-EDTA (the total volume was usually 19 ml) and appropriate aliquots then removed for heat coagulation, for precipitation with an equal volume of 1 *M* trichloroacetic acid (TCA), and for other purposes. The heat coagulation was achieved by placing 5-ml aliquots in test tubes immersed in a boiling water bath, usually for 10 minutes. At the end of this time, differences in turbidity were clearly apparent between control and auxin-treated proteins, the former being much more turbid. Where no obvious precipitates formed, the turbidities were measured in a Klett colorimeter equipped with a #42 blue filter. Where precipitates formed, they were centrifuged down, usually washed twice with 5-ml aliquots of distilled water, then pipetted onto tared weighing dishes, dried overnight in an oven and weighed.

In certain experiments, 14–16-day-old light grown peas (16-hr photoperiod, 1500 ft. candles intensity of mixed fluorescent and incandescent light, temperature

23°C) were used as a source of tissue. In this instance, about 160 five-mm stem sections (or half the number of 10-mm sections) were used per dish and the sections were incubated overnight in the light.[11]

Results.—Table 1 indicates typical results obtained with etiolated pea tissue material. The total protein, as obtained by TCA precipitation, is not altered by auxin treatment, but the fraction precipitable by boiling for 10 min is sharply reduced by the 2,4-D.

Table 2 shows a comparison of the activity of 2,4-D and of several other auxins and auxin analogs. Clearly, the most active auxins (IAA and 2,4-D) produce

TABLE 1

THE EFFECT OF 2,4-D ON THE PRECIPITABILITY OF ETIOLATED PEA STEM PROTEINS

2,4-D Molarity	Dry wt(mg) of Twice-Washed Precipitate—TCA Method	Dry wt(mg) of Twice-Washed Precipitate—Heat Method
0	5.9	4.6
10^{-5}	5.8	0.4

TABLE 2

EFFECT OF VARIOUS AUXINS AND AUXIN ANALOGS ON THE GROWTH AND HEAT COAGULABILITY OF PROTEINS OF ETIOLATED PEA EPICOTYL SECTIONS. ALL SUBSTANCES TESTED AT 10^{-5}M

Substance	Per cent Increase in—Length	Per cent Increase in—Fresh wt.	Dry wt. (mg) of Twice Washed Heat Coagulated Protein
Control (no addendum)	50	58	10.1
2,3,5-triiodobenzoic acid (TIBA)	40	54	6.3
2,4-D	82	106	1.2
p-Cl phenoxyisobutyric acid (PCIB)	42	45	9.9
Phenylacetic acid (PAA)	62	51	6.4
Indoleacetic acid (IAA)*	85	108	1.8

*Separate experiment.

TABLE 3

THE EFFECT OF VARIOUS CONCENTRATIONS OF 2,4-D ON THE GROWTH AND PROTEIN COAGULABILITY OF GREEN AND ETIOLATED PEA STEM SECTIONS

2,4-D Molarity	Green Sections—% Increase in Fresh wt.	Green Sections—Turbidity Reading	Green Sections—Mg Dry wt of Unwashed Heat-Coagulated Protein	Etiolated sections—% Increase in Fresh wt.	Etiolated sections—Mg Dry wt of Unwashed Heat Coagulated Protein
0 (initial control)	..	113	37.7	..	65.3
0 (final control)	33	136	27.5	55	53.6
10^{-6}	67	122	28.7	114	40.3
10^{-5}	93	87	20.1	130	21.9
10^{-4}	86	56	17.6	124	18.4

the greatest reduction in the weight of the protein coagulum; PCIB, which is an antiauxin analog of 2,4-D and slightly inhibitory to growth, produces no change in the protein precipitated; TIBA and PAA, both known to be slightly active as auxins,[12] produce smaller effects on the proteins.

Table 3 shows the effect of various concentrations of 2,4-D on the turbidity and heat coagulability of the proteins of green and etiolated pea stem sections. Clearly, there is an increasing effect on the proteins with increasing concentration of 2,4-D, even where the growth optimum may have been surpassed.

Subsequent experiments have revealed that: (1) 2,4-D is practically inactive when applied *in vitro* to control proteins, only slight effects being produced by the highest concentration (10^{-4} *M*) applied, (2) the difference between control and treated tissues resides mainly in the proteins of the non-particulate fraction, since boiling of the uncentrifuged homogenate yields differences only slightly greater than with all the particles removed, (3) the effect of 2,4-D on the proteins of etiolated pea stems can be detected in as little as four hours, and (4) in pea roots, where auxins induce marked growth inhibitions, effects similar to those described above are produced by overnight incubation with as little as 10^{-8}*M* 2,4-D.

Discussion.—It is at present impossible to estimate the physiological importance of the phenomenon described. A decision on this question will have to be deferred until the completion of extensive and lengthy tests on the relation of structure of auxin-type molecules to activity, on the relation of concentration of active molecules to activity, and on further kinetic experiments.

Whether or not the phenomenon is physiologically meaningful, it would appear to have considerable interest for the protein chemist. For example, it would be interesting to know whether the auxins produce this effect by attachment to the proteins, as previously indicated,[13] or by some indirect mechanism.

This phenomenon could explain the auxin-induced decreased viscosity of the cytoplasm described by Northen[2, 3] and the effects on cytoplasmic streaming observed by Sweeney and Thimann.[4–8] It might also be part of a general phenomenon which would explain the effects of auxin on wall[1] and nucleus,[9] as well as on the cytoplasm.

Summary.—Auxins induce a decrease in the heat coagulability of the proteins of growing pea stem cells to which they have been applied. The total protein content is not altered. The effect is produced mainly in the non-particulate phase of the cytoplasm. Auxin analogs which do not promote growth are less effective or completely ineffective. The effect is greatly reduced, or not produced at all when the active molecules are added *in vitro*.

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