

on minute amounts of the mediator liberated, etc.

There might be some relation between the role of histamine as a mediator of thermal stimulation and the increased histamine content in the perfusion liquid of isolated cat liver heated above 38°, reported by Rawlinson and Kellaway as an effect of cellular injury (5).

The experimental conditions described represent a convenient method of studying the influence of temperature variations on a reactive biological system, particularly from the standpoint of drug effect. Sudden temperature changes represent a stimulus for the smooth muscle, which contracts, probably as a result of the liberation of a histaminelike mediator. The contraction is inhibited by antihistaminic and antispastic drugs, but not by atropine.

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## Effects of an Exogenous Growth Regulator on Proteolytic Enzymes of the Soybean Plant<sup>1,2</sup>

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In recent years it has been shown that 2,4-dichlorophenoxyacetic acid (2,4-D) decreases the amount of protein nitrogen in the leaves and increases the level of this fraction in the stems and roots of treated plants (1-6). Accordingly, it was considered possible that this growth regulator affects proteolytic enzymes differently in the leaves and in the stems and roots. To test this hypothesis soybean plants were treated with 2,4-D and analyzed for proteinase and polypeptidase activity in leaves, stems, and roots.

Plants were grown in solution culture until they were 38-40 cm tall and had 6 fully expanded trifoliate leaves. At that stage the soybeans were treated by

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TABLE 1

EFFECT OF 2,4-D ON PROTEINASE AND POLYPEPTIDASE ACTIVITY IN LEAVES, STEMS, AND ROOTS OF SOYBEAN PLANTS

Days after treatment	Leaves		Stems		Roots	
	Controls	Treated	Controls	Treated	Controls	Treated
<i>Proteinase Activity*</i>						
0	1.97	—	0.14	—	0.43	—
1	1.88	1.48	.10	0.19	.59	1.01
3	1.68	1.47	.04	.52	0.42	1.28
5	1.82	1.22	0.09	0.34	—	—
<i>Polypeptidase Activity*</i>						
0	2.55	—	0.77	—	0.86	—
1	—	—	.84	0.90	1.16	1.25
3	2.49	2.35	.66	0.79	0.81	1.65
5	2.35	1.55	0.75	1.10	—	—

\* Measured as ml 0.05 N NaOH required to neutralize the increase in carboxyl groups after 24 hr incubation of plant enzyme extract with substrate of gelatin for proteinase activity or peptone for polypeptidase activity.

placing them in nutrient solutions containing 5 ppm of 2,4-D for an exposure period of 24 hr, after which the treated plants were returned to fresh nutrient solutions containing no 2,4-D.

Within 6 hr of the start of exposure to 2,4-D, twisting of stem tips and epinasty of petioles of treated plants were observed. By the third day after treatment, these symptoms were more pronounced, although all leaves were still completely turgid. By the fifth day, some of the leaves of the treated plants had wilted permanently and were just starting to turn dry. Most of the leaf tissue was still not dehydrated or dead, as indicated by the fact that the average percentage dry matter of all leaves of treated plants at this stage had reached only 19.5% as compared with 17.3% for the controls. Stems and bases of petioles of treated plants were definitely enlarged by this time.

Plants harvested before and after treatment were separated into the tissue fractions indicated in Table 1 and dried at 45° C. At each harvest reported in Table 1 all the leaves from 9 plants were composited into one sample for analysis. Each stem and root sample also represented all the respective tissue fraction from 9 plants. Glycerol extracts of the dried tissue were used for measurement of proteinase and polypeptidase activity according to methods described by Blagowestschenski and Melamed (7), Lauffer et al. (8), and Moundfield (9). Duplicate aliquots were analyzed, and determinations were repeated when good agreement between replicates was not obtained.

The results shown in Table 1 indicate that, with 2,4-D treatment, proteinase and polypeptidase activity decreased in the leaves by the end of the experiment, whereas in the stems and roots the activity increased considerably. The magnitude of the changes in proteolytic enzyme activity following treatment with 2,4-D indicates a significant influence of the growth regulator on this phase of nitrogen metabo-

lism. Obviously the activity of these two enzyme systems was not influenced by 2,4-D in the direction that might have been anticipated from observed changes in distribution of protein in leaves, stems, and roots following treatment of plants with 2,4-D (2). Possible interpretation of these findings will be presented elsewhere with further analyses of these plants.

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## Chlorogenic Acid a Possible Metabolite in the Terminal Oxidase System of the White Potato<sup>1</sup>

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Since chlorogenic acid occurs in a significant amount in the white potato (*Solanum tuberosum*), it may be involved in other functions aside from the protective action against invasion by *Streptomyces scabies* and other types of injury as reported by Johnson and Schaal (1). Boswell and Whiting (2) were among the first to demonstrate experimentally that polyphenolase is involved in the respiration of the potato tuber. They were able to concentrate a natural tyrosinase substrate from potato tuber. This substance gave a green color with  $\text{FeCl}_3$  which is characteristic of ortho-dihydroxy phenols. Upon adding it to respiring potato slices, they found increased rates of oxygen uptake and of carbon dioxide evolution. They were, however, not able to identify this substance. On the basis of the work of Johnson and Schaal, the indications are that it is chlorogenic acid.

Robinson and Nelson (3) contend that the active principle in potato juice which increases respiration is tyrosine. According to their view, tyrosine is oxidized to 3,4-dihydroxyphenylalanine (DOPA), which is the respiratory carrier. They estimated that 85% or more of the oxygen uptake may pass through this system.

The presence of chlorogenic acid in potatoes has been demonstrated by use of paper chromatography (1). However, no DOPA could be detected in potatoes by this method. Minute quantities of DOPA can be detected on a paper chromatogram by the use of Folin-Denis reagent or Pauly reagent (diazotized sul-

fanilic acid). The former gives a blue, and the latter a reddish-brown, color with DOPA. These reagents failed to show any DOPA in concentrated extracts prepared from potato flesh.

Rudskin and Nelson (4) found the natural polyphenolase substrate in the sweet potato (*Ipomoea batatas*) to be chlorogenic acid, and they concluded that chlorogenic acid and polyphenolase are involved in the terminal oxidase system of the sweet potato.

In view of the fact that chlorogenic acid is a natural substrate for tyrosinase (polyphenolase), and is present in greater quantities in the potato than DOPA, the author suggests that chlorogenic acid rather than DOPA is involved in the terminal oxidase system of the white potato.

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## Repeated Semiannual Spawning of Northern Oysters

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In Long Island Sound, as well as in ecologically similar areas, the spawning season of oysters, *Crassostrea virginica*, is confined to the period extending approximately from the last week of June until the beginning or middle of September. Thus the season is comparatively short and occurs once a year. It is the latter circumstance that suggested studies designed to determine whether the gonad development and spawning of the oysters were of the exogenous type—initiated and regulated by periodical seasonal changes of environment—or of the endogenous type—controlled by a pattern confined within the organism itself.

The question has been answered in part by experiments which showed that ripening of gonads in oysters could be achieved even in midwinter by placing the oysters for several weeks in warm water of about 20° C (1). It still remained uncertain, however, that this was not merely a case of precocious development of gonads which would make the oysters unable to undergo normal gametogenesis the following summer. To settle this an experiment was devised to find whether the oysters are able, under certain conditions, to accumulate and discharge spawn in a normal way at least twice a year, at intervals of about six months, and to do so for two or three successive years.

The experiment began in the spring of 1947 when a group of approximately 250 adult, individually numbered oysters was suspended on a float in Milford Harbor, Connecticut. By the middle of June, when the oysters reached ripeness, they were brought

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