This investigation deals with the physiological and biochemical studies on the mechanism of auxin action in rooting hypocotyl cuttings of Impatiens balsamina.

Hypocotyl cuttings were made from the seedlings by excising the root system. Two types of cuttings were made, those in which the cotyledons and apex were also excised and others in which these were left intact. These cuttings were cultured in test solutions in Petri-dishes and observations of the number of rooted cuttings and roots as well as the length of the hypocotyls were recorded at periodic intervals.

In all, 15 experiments were carried out. While experiment 1 deals with the study of the role of cotyledons and apex on the rooting response of cuttings cultured in media containing glucose and IAA and experiments 2 and 3
relate to the studies of the relative effectiveness of auxin when applied at different stages to the rooting cuttings. Experiment 4 deals with the study of the effect of temperature on the morphology, growth and rooting response of hypocotyl cuttings. The changes in the contents of chlorophyll, auxin, carbohydrates, DNA, RNA, proteins and amino acids were also studied in this experiment. Experiment 5 was carried out to study the effect of FUdR, Actinomycin-D, FU and Cycloheximide on the production of roots and the elongation of hypocotyls and experiment 6 to study the stage during the formation of root primordia which is affected by auxin (IAA) and the metabolic inhibitors (FUdR, act-D, FU and Cyc). Experiments 7, 8 and 9 deal with the interaction effects of the inhibitors with auxin and glucose and experiment 10 with the effect of pretreatment of cuttings with low and high concentrations of inhibitors on the rooting response as well as on the changes in the nucleic acid and protein contents. Experiments 11-13 were designed to study the effect of time and duration of application of IAA and metabolic inhibitors on the rooting response and its relationship with changes in the contents of carbohydrates, nucleic acids, proteins and amino acids and also with the activities of amylase, peroxidase and IAA-oxidase and experiments 14 and 15 with changes in the activities and isoenzyme patterns of peroxidase and IAA-oxidase as affected by IAA and Cycloheximide.
and their relationship with the rooting response.

The results obtained are as follows:

**Experiment 1**

1. More roots were produced on cuttings with than without cotyledons and apex (also experiments 7 and 9).
2. IAA increased rooting, the effect increasing with its concentration up to 1.0 mg/l and was more pronounced on cuttings with than without cotyledons and apex.
3. Glucose stimulated rooting, the number of roots on cuttings without cotyledons and apex in 2.0% glucose reaching a level almost equal to that on cuttings with intact cotyledons and apex cultured in water (also experiment 3).
4. The effectiveness of auxin varied with the endogenous level of nutrition and a precise balance between the two was essential for optimal root production. Thus, the most effective combinations were 0.5% glucose+10.0 mg/l IAA for the rooting of cuttings with and 1.0 mg/l glucose+1.0 mg/l IAA for those without cotyledons and apex.

**Experiments 2 and 3.**

5. The pretreatment of cuttings with IAA increased but that with water decreased the rooting of cuttings regardless of whether the cotyledons and apex were left intact or were excised, the effect increasing with the duration of the pretreatment.
6. Even a treatment with IAA for only 4 hr given during the first 10 hr in cuttings with intact cotyledons and apex
and initially in those without cotyledons and apex appreciably increased rooting.

**Experiment 4**

7. The germination of seeds was delayed at 31°C. The seedlings obtained were pale and had a poor root system while those at 25°C were dark green and with profuse root system.

8. More roots were produced on cuttings made from seedlings raised at 25°C than on those made from seedlings raised at 31°C.

9. The chlorophyll-, carbohydrate-, RNA-, protein- and amine acid- contents of the cotyledons and the hypocotyls were higher at 25°C than at 31°C. While the auxin and DNA contents of the cotyledons were higher at 31°C than at 25°C, the auxin content of the hypocotyl during the first 24 hr was higher at 25°C than at 31°C.

**Experiment 5**

10. IAA, FUDR, Act-D, FU and Cyc all suppressed the elongation of hypocotyls, the decrease being less in IAA and increased with the increasing concentration of each inhibitor (also experiments 11 and 12).

11. The roots produced on cuttings cultured in FUDR and Act-D were localised to the region immediately above the dipping part that decayed, while on cuttings cultured in FU and Cyc these were spread all over the surface (also experiments 11 and 12).
12. FUdR, Act-D, FU and Cyc all decreased rooting, the inhibitory effect increasing with concentration and being more marked in FUdR and Act-D than in FU and Cyc (also experiments 10, 11, 12 and 13). These results show that fresh synthesis of nucleic acids and proteins is necessary for rooting.

Experiment 6

13. The initiation of root primordia occurred at 48 hr on cuttings cultured in water and IAA, was delayed to 120 hr in those cultured in Cyc but was completely suppressed on cuttings cultured in either FUdR or Act-D.

14. The root primordia formed in Cyc were rounded in marked contrast to those with pointed tips in water and IAA.

Experiments 7 and 8

15. IAA was able to alleviate completely the inhibitory effect of 0.1 and 1.0 mg/l FUdR in cuttings with intact cotyledons and apex regardless of whether FUdR was given as a pre- or continual treatment. It was also able to overcome the inhibitory effect of 0.1 mg/l Act-D and the delaying effect of 1.0 and 10.0 mg/l FU.

Experiment 9

16. The inhibitory effect of antimetabolites was more pronounced on cuttings with than without cotyledons and apex and on those cultured in water, IAA and glucose each alone than in those cultured in IAA+glucose.
Experiment 10

17. The DNA-, RNA- and protein- contents of the cotyledons were much higher than those of the hypocotyls (also experiment 13).

18. Higher concentrations of FUdR, Act-D and Cyc decreased the DNA-, RNA- and protein- contents of the cotyledons as well as of the hypocotyls whether these were given as a pre- or continual treatment, the inhibitory effect in the former case being more in cuttings transferred to water than in those transferred to IAA.

19. Pretreatment with 0.1 mg/l Act-D stimulated the rooting of cuttings transferred subsequently to IAA.

20. Pretreatment with 0.1 mg/l Act-D increased the protein content of cotyledons in water at 48 hr but in IAA at 24 and 72 hr.

21. While continual treatment with Cyc depressed, 6 hr-pretreatment stimulated rooting regardless of whether the cuttings were subsequently transferred to water or to IAA.

22. Cyc decreased the RNA content of the cotyledons, the minimum reaching at 48 hr in 1.0 but at 24 hr in 100.0 mg/l Cyc.

23. Pretreatment with 1.0 mg/l Cyc increased the protein content of the hypocotyls at 48 and 72 hr in cuttings transferred to IAA.

Experiments 11 and 12

24. The inhibitory effect of FUdR and Act-D was most
pronounced when applied during the first twelve hours and decreased with the delay in their application (also experiment 13).

25. The inhibitory effect of 12 hr-FU treatment was most pronounced when applied during 0-12 or 6-18 hr than when applied later.

26. 12 hr-Cyc treatment decreased the number of roots when given during 12-24 hr but increased it when given at any other stage. 6 hr-Cyc treatment during 0-6 or 24-30 hr also increased the number of roots on cuttings cultured in IAA.

27. FUdR, Act-D, FU, Cyc and IAA all suppressed the elongation of roots, as well as the hypocotyls.

Experiment 13

28. **Carbohydrate content**

   (a) The carbohydrate content of the cotyledons was lower but that of the hypocotyls higher in IAA than in water.

   (b) While FUdR increased the carbohydrate content of the cotyledons and decreased that of the hypocotyls, Act-D decreased the content of both.

   (c) The carbohydrate content of the cotyledons was higher in Cyc than in water after 6 hr.

   (d) 6 hr-Cyc treatment at any stage during 6-48 hr decreased the carbohydrate content of the hypocotyls.

29. **RNA content**

   (a) IAA increased the RNA content of the cotyledons
as well as of the hypocotyls over that in water during the first 12 hr.

(b) 6 hr-treatment with IAA during 18-24 and 42-48 hr also increased the RNA content of the cotyledons.

(c) Act-D treatment during the first 12 hr decreased the RNA content of the cotyledons as well as of the hypocotyls.

(d) Cyc increased the RNA content of the cotyledons till 24 hr but the content of the hypocotyle till 48 hr.

30. Protein content

(a) IAA increased the protein content of the cotyledons as well as of the hypocotyls, the continual treatment being more effective than that for only 6 hr and the stimulatory effect of the latter decreasing with the delay in its application.

(b) FudR and Act-D decreased the protein content of the cotyledons as well as of the hypocotyls. The inhibitory effect of 6 hr-Act-D treatment was most pronounced when given during 0-6 hr.

(c) 6 hr-Cyc treatment during 0-6 hr increased but at later stages decreased the protein content of the cotyledons.

(d) Cyc decreased the protein content of the hypocotyles at all stages.

31. Amino acid content

(a) IAA increased the amino acid content of the hypocotyls during the first 6 hr but decreased it subsequently.
(b) FUdR increased the amino acid content of the cotyledons but decreased that of the hypocotyls during the first 24 hr.

(c) Act-D decreased the amino acid content of the cotyledons as well as of the hypocotyls till 24 hr.

32. Amylase activity
(a) Amylase activity of the cotyledons was higher than that of the hypocotyls.

(b) IAA increased the activity of amylase, the increase being considerable even with 6 hr treatment given during 18-24 hr.

(c) While FUdR and Act-D decreased the amylase activity of the cotyledons Cyc increased it but all the three antimetabolites decreased the activity of the hypocotyls.

33. Peroxidase and IAA-oxidase activity
(a) IAA increased the activity of peroxidase.

(b) IAA increased also the IAA-oxidase activity during the first 24 hr.

(c) FUdR and Act-D decreased IAA-oxidase activity.

(d) Cyc increased both peroxidase and IAA-oxidase activity during the first 6 hr but decreased it subsequently.

Experiment 14

34. Pretreatment of cuttings with Cyc for 24 hr stimulated rooting of cuttings subsequently cultured in IAA.

35. New isoenzymes of peroxidase were induced in cuttings
cultured in Cyc as well as in those pretreated with it for 24 hr regardless of whether these were subsequently transferred to water or to IAA.

36. The elution profiles of the isoenzymes showed that the peaks of IAA-oxidase activity coincided with those of peroxidase activity corresponding to the isoperoxidase bands on the gels.

Experiment 15

37. Rooting of etiolated hypocotyl cuttings was higher in IAA+glucose than in water.

38. The peroxidase and IAA-oxidase activities increased in IAA+glucose alone or together with 1.0 mg/l Cyc but not with 10.0 mg/l Cyc.

39. New isoenzymes of both peroxidase and IAA-oxidase were induced in IAA+glucose and IAA+glucose+1.0 mg/l Cyc.

40. 10.0 mg/l Cyc used together with IAA+glucose delayed/suppressed the induction of new isoperoxidase and IAA-oxidases.

Paradoxical effects of antimetabolites (experiments 5, 7, 8, 9, 10, 13 and 14).

Treatment with low concentrations of Act-D (7, 8 and 9), FU (9) and Cyc (5, 7, 8, 9 and 14) stimulated rooting of cuttings subsequently cultured in water or IAA. 6 hr-pretreatment with even higher concentration of Cyc (13) increased the number of roots over control.

These stimulatory effects are discussed in the light of existing literature.