THE EFFECT OF ETHIONINE ON SOME PLANT GROWTH SYSTEMS

by

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A THESIS

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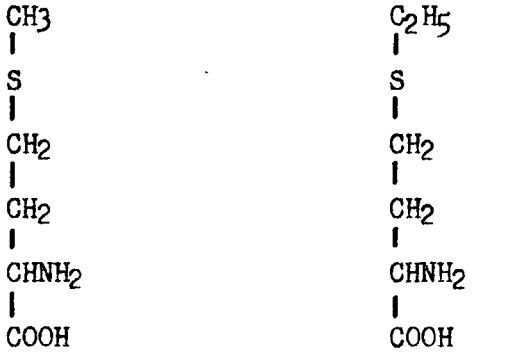
1.0 INTRODUCTION

In order for a chemical compound to be defined as an antimetabolite it must be structurally similar to the metabolite and inhibit a biochemical pathway involving the metabolite. By definition this inhibition must be reversed by the metabolite. Ethionine is a synthetic compound which is structurally related to methionine in that it is formed by replacing the terminal methyl group of methionine by an ethyl group (Figure 1).



Methionine Ethionine

Figure 1.

Ethionine has been shown to be an antimetabolite to methionine in a number of biological systems involving animals and microorganisms (Martin, 1951; Wooley, 1952; Rabinovitz et al, 1954). Until recently its effects on higher plants have not been known. However, Boll (unpublished data), found that isolated tomato roots grown in sterile culture exhibit a response to ethionine similar to those reported with microorganisms and animals. He also has found that concentrations of ethionine

and of ethionine plus methionine have profound effects upon the morphology of the root. This thesis concerns the effect of ethionine on some plant growth systems other than the excised tomato root.

2.0 REVIEW OF THE SUBJECT

This section will include: (1) a general description of antimetabolites and their mode of action; (2) a brief description of inhibition analysis as a technique which has arisen from the study of antimetabolites; (3) a survey, in tabular form, of the effects of antimetabolites on plants and some comments on the uses to which antimetabolites have been applied in plant studies; (4) a review of the effects of ethionine on tomato roots when they are excised and grown under sterile conditions and the possible significance of these effects.

2.1 THE MEANING AND MODE OF ACTION OF ANTIMETABOLITES

The inhibition of enzymatic reactions by substances structurally related to the substrate, and competitive antagonism between the substrate and inhibitor, had been known for some years when Woods (1940) showed that the bacteriostatic action of the sulfonamide drugs could be overcome by p-aminobenzoic acid (PABA). Woods recognized a competitive antagonism between PABA and the drug in that when various amounts of the drug were supplied to the sensitive bacteria the inhibition of growth was overcome by the addition of a proportionate amount of PABA. Woods' discovery initiated a broad field of research concerning the biological effects of antimetabolites.

One objective in the study of antimetabolites was originally defined by Fildes (1940) who suggested that antimetabolites other than sulfonamides could be devised and used as chemotherapeutic agents. These studies have

not proved as profitable as their proponent may have wished although there are some examples of success in this field e.g. the production of remissions in children with acute leukemia by aminopterin which is a folic acid analogue (Burchenal, 1955).

Despite the lack of widespread success in using antimetabolites in chemotherapy the research on antimetabolites has yielded considerable new biochemical information. This information includes: (1) the discovery of new metabolites, (2) attributing new functions to previously recognized metabolites, (3) tracing metabolic pathways, (4) determination of the existence and nature of individual enzyme reactions and (5) the investigation of the active centers of enzyme action. The use of antimetabolites has also proven useful in localizing the role of various metabolites in morphological development and thus in studying the physiological processes involved with growth itself. The principles and possibilities of research involving the use of antimetabolites are discussed in considerable detail by Martin (1951) and Wooley (1952).

Antimetabolites may be naturally occuring compounds such as the Pseudomonas bacterial toxin (Wooley 1955) or they may be synthetic compounds such as ethionine (Dyer, 1938). Both of these antimetabolites are structural analogues to the sulphur amino acid methionine.

An antimetabolite is pictured as acting by excluding a structurally related metabolite from combining with its normal specific reactant. This reactant is usually an enzyme so that the antimetabolite, by preferentially attaching itself to the enzyme, excludes the metabolite and thus prevents the formation of an enzyme substrate (metabolite) complex. The antimetabolite can replace the metabolite because it possesses most of

the structural features which allow the metabolite to react. The result of this exclusion of the metabolite is manifested in living organisms or their parts by showing signs of deficiency of the metabolite usually by limiting the growth of the organism. As has been mentioned previously, the addition of an appropriate amount of the corresponding metabolite will antagonize the inhibition caused by the antimetabolite.

Antimetabolites are either competitive or non-competitive in their inhibition of biological systems. In the case of the competitive type of inhibition, it is assumed that both the analogue, or some derivative thereof, and the metabolite compete for the same reaction site on an enzyme surface. The reaction can be represented as follows:

$$E + S \rightleftharpoons ES \rightarrow E + P$$

where E=enzyme, S=metabolite or substrate, ES=enzyme substrate complex and P=product. As a result of this substitution the enzyme may cease to function or the analogue-enzyme complex undergoes dissociation into enzyme plus analogue product, instead of metabolite product, and the "normal" reaction is prevented from occuring. Occasionally, the analogue will replace the metabolite and the reaction will produce an analogue-product which can replace the metabolite product but when this occurs the analogue is usually less effective than the metabolite and a much higher concentration of the analogue, as opposed to the metabolite, is necessary to achieve the same biological activity.

In the case of the non-competitive type of inhibition, between substrate and analogue, it is assumed that the analogue or inhibitor reacts with a catalytically inactive site on the enzyme surface and this type of inhibition may be expressed as follows:

$$ES + I \rightleftharpoons ESI = \text{inactive.}$$

In the above equation ES=enzyme substrate, I=inhibitor or analogue, and ESI=inactive inhibitor enzyme complex.

Experimental methods for identifying competitive and non-competitive inhibitions are given by Williams et al (1950) and Wooley (1952). The identification may be made by studying the inhibition index of the reactions involved. The inhibition index is defined as being a constant ratio of concentration of analogue to that of the metabolite which is required for a defined degree of inhibition, obtained during a defined experimental period, when only the concentration of inhibitor and metabolite are varied. If the index remains the same over a considerable concentration range then the relationship is said to be a competitive one. If the index does not remain the same over a considerable concentration range then the relationship is non-competitive. A competitive inhibition is interpreted as showing that the antagonist (metabolite) is a participant or precursor of such a participant in the metabolic step inhibited by the antimetabolite. A non-competitive inhibition is, generally, interpreted as showing that the antagonist is a product, immediate or remote, of the metabolic step inhibited by the antimetabolite, unless the antagonist is known to be the substrate of the enzyme (see above).

However, on theoretical grounds there are other biochemically related substances which can prevent, non-competitively, the antimetabolite toxicity. Included among these substances are the following types: 1) those which increase the concentration of the metabolite synthesized by the biological system; 2)those exerting a sparing effect on the product of the inhibited enzyme system; 3) compounds which increase the effective enzyme

concentration; 4) a group of miscellaneous substances including compounds affecting the rate of destruction of either the inhibitor or metabolite and 5) those non-competitive agents termed "secondary antagonists". Work and Work (1948) have classified the secondary antagonists into the following four groups: a) essential metabolites which are displaced; b) substances which increase the growth rate and so mask antimetabolite action; c) compounds which combine with an antimetabolite; and d) metabolites secondary to some prime reaction inhibited by the antimetabolite. These last named are considered to be the true secondary antagonists.

2.2 INHIBITION ANALYSIS

Studies on the modes of action of the five groups of compounds mentioned above have been made by employing the techniques of "inhibition analysis". An example of a complex situation to which the technique of "inhibition analysis" has been applied is the example from which the techniques actually arose i.e. the relationships involving sulfanilamide and methionine and the purines. The techniques were first put forward by Harris and Kohn (1941) and were later elaborated and extended by Shive and Roberts (1946) and Shive (1950). In brief, inhibition analysis is based upon the hypothesis that the metabolite is a substrate for more than one enzyme. Of this family of enzymes only one will be susceptible to combination with the antimetabolite at one time or at one concentration of the metabolite. By supplying the product, immediate or remote, of each enzyme system as it becomes inhibited, the effect of the antimetabolite on that particular enzyme system is no longer of consequence. Thus the

substances which successfully erase these enzyme inhibitions and the order in which they do so help to reveal the metabolic pathways involving the metabolite. The inhibition index is also used to detect the postulated products of the reactions in inhibition analysis. If the index is greater when it is determined in the presence of the structurally dissimilar antagonist, the antagonist is concluded to be a product of the reaction. Mathematical equations have been developed which express the ideas involved in this analysis (Shive and Roberts, 1946 and Shive 1950). It must be realized that the validity of the conclusions derived from investigations employing inhibition analysis, are dependent upon the validity of the assumptions inherent in the experimental methods. Nevertheless, the results of inhibition analysis have been used by numerous investigators to postulate details about the route of formation of nucleic acid derivatives, amino acids and other metabolites in microorganisms and animals.

2.3 THE EFFECT OF ANTIMETABOLITES ON PLANTS

Although the early research involving antimetabolites and their effects on biological systems was done in connection with microorganisms and animals, their effects on higher plants, for the most part, have been studied only recently. This section contains a general section dealing with the literature on the effect of antimetabolites on plants.

2.31 TABULAR REVIEW OF THE LITERATURE

In the studies involved with testing the effects of antimetabolites on plants either whole plants, their isolated organs or isolated tissues have been used, under sterile or non-sterile conditions as indicated in Tables I to VII.

Two kinds of information may be derived by observing the effects of antimetabolites on higher plants or their isolated parts. The use of antimetabolites as a tool for studying metabolic inter-relationships is accomplished by studying the nutritional conditions required to remove metabolic blocks produced by antimetabolites. Effective antimetabolites may also be used to study the physiological function of a metabolite by controlling the effective level of the metabolite, or related substances, and observing the changes in known physiological processes such as growth or changes in morphology.

Tables I to VII represent a review of the literature involving the use of antimetabolites to study plant processes including virus and other pathological growths. This survey is restricted to antimetabolites of vitamins, nucleic acid derivatives, amino acids and inorganic ions. 'Anti-auxins' are omitted intentionally from this survey. It will be noted that for each of these groups of antimetabolites the data has been divided into two tables. Thus investigations revealing biochemical information are listed separately from those which reveal information pertaining to physiological processes.

Some of the compounds, included in the tables may not be antimetabolites but are included because of their structural similarity to a metabolite. Thus, although isonicotinic acid hydrazide (Tables I and II) is not proven to be an antimetabolite it is included here because of its structural similarity to niacin — which would tend to suggest that it is an antimetabolite to that vitamin. Another example of a compound which is included but has not been proven to be an antimetabolite is azaserine. In a recent letter, Dr. Norman at the University of Michigan, who has worked

with azaserine, stated that he considers this compound to be an antimetabolite to serine. For this reason azaserine has been placed in the tables containing antimetabolites to amino acids (Tables V and VI).

TABLE I ANTIMETABOLITES TO VITAMINS (BIOCHEMICAL DATA)

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#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCE
1	p-amino-benzene -sulphonamide	Isolated tomato roots grown in vitro	p-aminobenzoic acid (PABA)		It is shown that isolated tomato roots may synthesize PABA.	Bonner, 1942
2	desoxypyridoxine (DOP)	Germinating pulse seeds	pyridoxine (vitamin B6)		Nicotinic and ascorbic acid metabolism was studied.	Shanmuga Sandaram et al, 1951
3	ditto	Excised tomato roots in culture	pyridoxine	competitive	The data supports the view that the antagonism between pyridoxine and DOP involves the prior conversion of DOP to DOP phosphate, which then competes with pyridoxal phosphate or pyridoxamine phosphate.	Boll, 1954c
4	2,4,-diamino-9,10-dimethyl-pteroylglutamic acid =(Aminopterin) (Ap)	Germination of Phaseolus mungo L.	pteroylglutamic acid = folic acid (PGA)		Ap inhibits nicotinic acid synthesis.	Shanmuga Sandaram and Sarma, 1954
5	ditto	ditto	PGA		Ap affects amino acid changes. Histidine, methionine, and tyrosine levels are affected but not that of threonine.	Sivaramakrishnan and Sarma, 1954
6	ditto	Pea plantlets	PGA		Ap partially inhibits chlorophyll and carotenoid formations. PGA partially reverses this inhibition.	Schopfer and Grob, 1954
7	dimethylbenzoyl sulfamide (DMBS)	Isolated pea roots in sterile culture	thymine cytosine uracil guanine adenine phosphate		The data suggest that in plants PABA or some derivative of it is involved in the biosynthesis of pyrimidines or derivatives of them.	Anker, 1951
8	isonicotinic acid hydrazide (INH)	Pea plantlets			INH at 2.4 mg. in 20 cm.^{3} causes 50% inhibition of carotenoid formation.	Schopfer, Grob and Besson, 1952 II
9	ditto	(a)Oat seedlings (b)Bean seedlings (c)Sugar beet seedlings			Chlorophyll formation is not affected by INH. 1.2% INH (87 mM per ml.) depressed slightly, leaf catalase and phosphatase activities.	Wort, 1954

TABLE I (CONTINUED)

10	W-methylpantothenic acid	Germination of Phaseolus mungo			The antimetabolite has no influence on the biosynthesis of nicotinic acid	Shanmuga Sandaram and Sarma, 1954
11	neopyrithiamine	ditto	thiamine (vitamin B_1)		The analogue produces an accumulation of glutamic acid and a fall in the aspartic acid synthesis. (Normally, the reverse is true).	Sivaramakrishnan and Sarma, 1954
12	sulfanilamide	Growth of garden cress plants			Growth of the plants was inhibited.	Fourneau et al, 1936
13	ditto	Isolated tomato roots grown in vitro	PABA		PABA almost completely reverses the inhibition of growth caused by the antimetabolite. It is shown that the isolated roots may synthesize PABA.	Bonner, 1942
14	ditto	Roots of Pisum sativium L. grown on agar	PABA	competitive	The data fit in with the Woods-Fildes theory (1940) and suggest that PABA is an essential metabolite in plants as well as in microorganisims.	Wielding, 1943
15	ditto	Roots of Pisum sativum in sterile culture	PABA guanine adenylic acid ribonucleic acid	competitive	This data parallels the work of Woods (1940) with sulfanilamide in microorganisims. It was found that sulfanilamide increased the rate of decomposition of ribonucleic acid.	Schopfer and Anker, 1949
16	ditto	Growth of flax seedlings	PABA pteroic acid p-aminobenzoyl glutamic acid (PABG) PGA	competitive competitive competitive	Sulfanilamide at 4.4 log molar units produces 50% inhibition of the seedling growth. The results are interpreted as follows:- PABA acts as a precursor of the inhibited reaction. and PGA acts as a product of this reaction. It is thought that the antimetabolite interferes with the conversion of PABA to PGA; pteroic acid and PABG act by prior conversion to PABA.	Moore, 1953b
17	ditto	Germination of Phaseolus mungo	PABA PGA		The data suggest a metabolic relationship between methionine and histidine and that PABA may be directly concerned in histidine metabolism. PABA and PGA appear to be involved in serine and methionine metabolism but not in the metabolism of threonine.	Sivaramakrishnan and Sarma, 1953

TABLE I (CONTINUED)

18	ditto	Growth of excised tomato roots in sterile culture	PABA PGA methionine	competitive competitive	The data provide good evidence that PABA or PGA, or some related substance synthesized from one of them is involved in the metabolism of the excised tomato root. From the data it is concluded that PABA is a precursor of or a participant in the metabolic step blocked by sulfanilamide. These results are in agreement with those generally obtained with microorganisims. The results indicating a competitive antagonism between PGA and sulfanilamide are not in agreement with those generally obtained with microorganisims. It is apparent that the folic acid metabolism of this tomato roots is unlikely to be identical with that suggested for the majority of the microorganisims studied to date.	Boll, 1955
19	ditto	ditto	thymine cytidine cytidylic acid uridylic acid		The data suggest that in plants PABA or some derivative of it is involved in the biosynthesis of pyrimidines or derivatives of them.	Boll, unpublished
20	sulfapyridine	Isolated tomato roots grown in vitro	PABA	competitive	Reversal of the inhibition is almost complete. The data suggest that these isolated roots synthesize PABA.	Bonner, 1942
21	ditto	Roots of Pisum sativum grown on agar	PABA	competitive	Another example of a sulfonamide which confirms the antagonistic effect of PABA in higher plants. The data suggest that PABA is an essential metabolite in higher plants as well as in microorganisims.	Wielding, 1943
22	sulfathiazole	Isolated tomato roots in sterile culture	PABA	competitive	At 2 mg. per litre sulfathiazole a 50% inhibition of root growth occured. The inhibition was markedly decreased by 100 times smaller quantities of PABA.	Bonner, 1942
23	δ-(3,4-ureylene-cyclohexyl) butyric acid	Germination of Phaseolus mungo	biotin (Vitamin H)		The analogue inhibits the biosynthesis of nicotinic acid. The results obtained suggest that biotin may participate in tryptophane breakdown between tryptophane and kynuremine.	Shanmuga Sandaram and Sarma, 1954
24	ditto	ditto	ditto		The analogue inhibits the usual fall in glutamic acid and increase in aspartic acid; as a result glutamic acid is accumulated while aspartic acid decreases.	Sivaramakrishnan and Sarma, 1954

TABLE II ANTIMETABOLITES TO VITAMINS (PHYSIOLOGICAL DATA)

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#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCE
1	2-acetylamino-1,3,4-thiodiazole-5-sulfonamide	Growth of Lemna minor L.			10^{-3} M was the minimum concentration which inhibited growth.	Fromm and O'Donnell, 1955
2	5-acetylpyridine	Growth of Arundo donax L.	nicotinic acid (niacin)		Under normal conditions growth is controlled by niacin. The antivitamin increased growth when applied.	Vittoria and Ascarelli, 1956
3	N^1-acetylsulfanilamide	Growth of Lemna minor			10^{-4} M was the minimum concentration which inhibited growth. 10^{-2} M killed the plantlets.	Fromm and O'Donnell, 1955
4	N^4-acetylsulfanilamide	ditto			10^{-2} M was the minimum concentration which inhibited growth.	ditto
5	p-aminobenzene sulfonamide (PABS)	Growth of isolated tomato roots in sterile culture	p-aminobenzoic acid (PABA)			Bonner, 1942
6	ditto	Growth of main roots and production of lateral roots in Allium cepa L.	PABA		PABA at 1/200,000 plus PABA at 1/2,000 causes less inhibition of the main root and sprouting of the lateral roots than PABS alone at 1/2,000.	Stoll, 1943
7	ditto	Germination and development of wheat seeds grown in soil and in sand	PABA		PABS when powdered on seeds sown in soil caused a reduction in germination and a stunting of those plantlets which did emerge. When the seeds were presoaked in PABS and then sown in sand they germinated but the coleoptiles and roots were stunted. The addition of PABA in the aqueous solution cancelled this effect.	Brian, 1944
8	4-amino-9,N^{10} dimethylpteroylglutamic acid = (A-denopterin)	Growth of tumors induced on carrot tissue by crown gall bacteria			A-denopterin completely suppressed the growth of the tumors.	deRopp, 1949

TABLE II (CONTINUED)

9	ditto	i)Tumor tissue on stem fragments of Garden Chrysanthemum var. Golden treasure Healthy tissues of: ii)Sunflower crowngall (bacteria free) (iii)Excised tomato roots iv)Excised sunflower embryos	pteroyl glutamic acid (PGA) PGA		i)Growth was actively inhibited. ii)Growth was completely suppressed at 10-100 mg. per litre. iii) " " " " " 1 mg. per litre. iv) " " " " " 10-100 mg. per litre. Note No specific inhibition occured in healthy as opposed to tumor tissue.	deRopp, 1950
10	ditto	a)Tumor tissue produced on stem fragments of Garden Chrysanthemum by crown gall bacteria b)Excised tomato roots c)Bacteria free crown gall d)Sunflower embryos			a)Growth was inhibited. b)At 1 to 10 mg. per litre growth was inhibited. c)Less sensitive than a) or b) to the antimetabolite. d)ditto	deRopp, 1951
11	4-amino-α-glutamyl -α glutamyl glutamic acid (A-teropterin)	i)-iv) See #9 above	See #9 above		i)Growth was inhibited. ii)-iv)Growth was inhibited one-tenth as much as it was with A-methopterin, A-ninopterin, and A-denopterin. Note No specific inhibition occured in healthy as opposed to tumor tissue.	deRopp, 1950
12	ditto	a)-d) See #10 above			a)-d)See #10 above.	deRopp, 1951
13	4-amino-N^{10}-methyl pteroyl-glutamic acid = (A-methopterin)	See #8 above			Growth of the tumors was completely suppressed.	deRopp, 1949
14	ditto	i)-iv) See #9 above			i)-iv)See #9 above.	deRopp, 1950
15	ditto	a)-d) See #10 above			a)-d)See #10 above.	deRopp, 1951

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16	4-amino-9 methylpteroyl-glutamic acid (A-ninopterin)	See #8 above.			Growth of the tumors was completely suppressed.	deRopp, 1949
17	ditto	i)-iv)See #9 above	See #9 above		i)-iv)See #9 above.	deRopp, 1950
18	ditto	a)-d)See #10 above			a)-d)See #10 above.	deRopp, 1951
19	p-aminophenyl sulfamide	Root growth of Pisum sativum seedlings Root growth of Lupinus sp. seedlings	PABA		The antimetabolite caused all the submerged parts of the root to turn brown but in spite of this many lateral roots were produced. A stimulation of lateral root production occurs.	Mangenot and Carpentier, 1942
20	ditto	Germination of Linum usitatissimum L.	PABA		The antimetabolite at 200 p.p.m. inhibits germination.	Hazard, 1944
21	4-aminopteroyl aspartic acid =(amino anifol)	i)-iv)See #9 above	See #9 above		i)-iv)See #9 above.	deRopp, 1950
22	ditto	a)-d)See #10 above			a)-d)See #10 above.	deRopp, 1951
23	4-aminopteroyl glutamic acid (Aminopterin)	See #8 above			Growth of the tumors was completely suppressed.	deRopp, 1949
24	ditto	i)-iv)See #9 above	See #9 above		i)-iv)See #11 above.	deRopp, 1950
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24	ditto	ditto	ditto		The analogue inhibits the usual fall in glutamic acid and increase in aspartic acid; as a result glutamic acid is accumulated while aspartic acid decreases.	Sivaramakrishnan and Sarma, 1954

TABLE II ANTIMETABOLITES TO VITAMINS (PHYSIOLOGICAL DATA)

#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCE
1	2-acetylamino-1,3,4-thiodiazole-5-sulfonamide	Growth of Lemna minor L.			10^{-3} M was the minimum concentration which inhibited growth.	Fromm and O'Donnell, 1955
2	5-acetylpyridine	Growth of Arundo donax L.	nicotinic acid (niacin)		Under normal conditions growth is controlled by niacin. The antivitamin increased growth when applied.	Vittoria and Ascarelli, 1956
3	N^1-acetylsulfanilamide	Growth of Lemna minor			10^{-4} M was the minimum concentration which inhibited growth. 10^{-2} M killed the plantlets.	Fromm and O'Donnell, 1955
4	N^4-acetylsulfanilamide	ditto			10^{-2} M was the minimum concentration which inhibited growth.	ditto
5	p-aminobenzene sulfonamide (PABS)	Growth of isolated tomato roots in sterile culture	p-aminobenzoic acid (PABA)			Bonner, 1942
6	ditto	Growth of main roots and production of lateral roots in Allium cepa L.	PABA		PABA at 1/200,000 plus PABA at 1/2,000 causes less inhibition of the main root and sprouting of the lateral roots than PABS alone at 1/2,000.	Stoll, 1943
7	ditto	Germination and development of wheat seeds grown in soil and in sand	PABA		PABS when powdered on seeds sown in soil caused a reduction in germination and a stunting of those plantlets which did emerge. When the seeds were presoaked in PABS and then sown in sand they germinated but the coleoptiles and roots were stunted. The addition of PABA in the aqueous solution cancelled this effect.	Brian, 1944
8	4-amino-9,N^{10} dimethylpteroylglutamic acid = (A-denopterin)	Growth of tumors induced on carrot tissue by crown gall bacteria			A-denopterin completely suppressed the growth of the tumors.	deRopp, 1949

TABLE II (CONTINUED)

9	ditto	i)Tumor tissue on stem fragments of Garden Chrysanthemum var. Golden treasure Healthy tissues of: ii)Sunflower crowngall (bacteria free) (iii)Excised tomato roots iv)Excised sunflower embryos	pteroyl glutamic acid (PGA) PGA		i)Growth was actively inhibited. ii)Growth was completely suppressed at 10-100 mg. per litre. iii) " " " " " 1 mg. per litre. iv) " " " " " 10-100 mg. per litre. Note No specific inhibition occured in healthy as opposed to tumor tissue.	deRopp, 1950
10	ditto	a)Tumor tissue produced on stem fragments of Garden Chrysanthemum by crown gall bacteria b)Excised tomato roots c)Bacteria free crown gall d)Sunflower embryos			a)Growth was inhibited. b)At 1 to 10 mg. per litre growth was inhibited. c)Less sensitive than a) or b) to the antimetabolite. d)ditto	deRopp, 1951
11	4-amino-α-glutamyl -α glutamyl glutamic acid (A-teropterin)	i)-iv) See #9 above	See #9 above		i)Growth was inhibited. ii)-iv)Growth was inhibited one-tenth as much as it was with A-methopterin, A-ninopterin, and A-denopterin. Note No specific inhibition occured in healthy as opposed to tumor tissue.	deRopp, 1950
12	ditto	a)-d) See #10 above			a)-d)See #10 above.	deRopp, 1951
13	4-amino-N^{10}-methyl pteroyl-glutamic acid = (A-methopterin)	See #8 above			Growth of the tumors was completely suppressed.	deRopp, 1949
14	ditto	i)-iv) See #9 above			i)-iv)See #9 above.	deRopp, 1950
15	ditto	a)-d) See #10 above			a)-d)See #10 above.	deRopp, 1951

TABLE II (CONTINUED)

16	4-amino-9 methylpteroyl-glutamic acid (A-ninopterin)	See #8 above.			Growth of the tumors was completely suppressed.	deRopp, 1949
17	ditto	i)-iv)See #9 above	See #9 above		i)-iv)See #9 above.	deRopp, 1950
18	ditto	a)-d)See #10 above			a)-d)See #10 above.	deRopp, 1951
19	p-aminophenyl sulfamide	Root growth of Pisum sativum seedlings Root growth of Lupinus sp. seedlings	PABA		The antimetabolite caused all the submerged parts of the root to turn brown but in spite of this many lateral roots were produced. A stimulation of lateral root production occurs.	Mangenot and Carpentier, 1942
20	ditto	Germination of Linum usitatissimum L.	PABA		The antimetabolite at 200 p.p.m. inhibits germination.	Hazard, 1944
21	4-aminopteroyl aspartic acid =(amino anifol)	i)-iv)See #9 above	See #9 above		i)-iv)See #9 above.	deRopp, 1950
22	ditto	a)-d)See #10 above			a)-d)See #10 above.	deRopp, 1951
23	4-aminopteroyl glutamic acid (Aminopterin)	See #8 above			Growth of the tumors was completely suppressed.	deRopp, 1949
24	ditto	i)-iv)See #9 above	See #9 above		i)-iv)See #11 above.	deRopp, 1950
25	ditto	a)-d)See #10 above			a)-d)See #10 above.	deRopp, 1951

TABLE II (CONTINUED)

26	desoxypyridoxin (DOP)	Growth of excised tomato roots	pyridoxin	competitive	DOP inhibits growth. However, growth of the main axis was greater in the presence of DOP and pyridoxin in the ratio of 10:3 than with any concentration of pyridoxin alone. The function of pyridoxin in some mechanism (or mechanisms) of growth is indicated here.	Boll, 1954c
27	N^1,N^4-diacetyl sulfanilamide	Growth of Lemna minor			10^{-2} M killed the plantlets. 10^{-3} M was the lowest concentration which inhibited growth.	Fromm and O'Donnell, 1955
28	2,4-diamino-9, 10-dimethyl-pteroylglutamic acid	i)Development of pea plantlets in synthetic media ii)Isolated pea roots in vitro			i)Growth of the primary roots and production of lateral roots are inhibited. ii)Cell division is inhibited in these primary roots.	Schopfer and Grob, 1954
29	dimethylbenzoyl sulfamide (DMBS)	Growth of roots of Pisum sativum in pure culture	PABA purines except adenine		At a concentration of 1:6 x 10^{-7} M of DMBS to PABA there is a 70% increase in lateral number produced over the control number. DMBS inhibition of root growth is reversed by purines (all except adenine) at 50 times the concentration of DMBS.	Anker, 1951
30	homothiamine-glycol	Growth of pea roots in sterile culture	thymine		Root growth is inhibited. This antimetabolite is 373:7 times less active than neopyrithiamine is in inhibiting root growth.	Schopfer et al, 1951
31	isonicotinic acid hydrazide (INH)	i)Pea root growth ii)Growth of pea plantlets in 20 $cm.^3$	IAA		i)50% inhibition occurs with 785 gamma INH.in 20 $cm.^3$. ii)50% inhibition of the length increase in the aerial parts occurs at 35.6 mg. INH. Primary root elongation is inhibited 50% at 400 gamma INH. The weight increase of the lateral roots is inhibited 50% by 410 gamma INH.	Schopfer, Grob and Besson, 1952
32	ditto	Germination of seeds and development of: a)Raphanus sativus L. b)Triticum vulgare Vill. c)Lupinus albus L.			0.4% INH completely prevented germination of a,b, and c. Seedling development of a,b, and c was inhibited at 200, 50 and 10 p.p.m. respectively.	Bustinza and Santamaria, 1953

TABLE II (CONTINUED)

33	ditto	Growth of Lemna minor			Growth was stimulated by 1 to 20 p.p.m. INH. The effects of higher concentrations were not recorded.	Nickell and Findlay, 1954
34	ditto	Growth of seedlings of certain higher plants			INH was applied to the foliage. General retardations of topgrowth was observed with INH concentrations of 0.4 to 1.6%. The same concentrations when applied to the soil caused stunting except at the 1.6% level, which was lethal to several species.	Wort, 1954
35	neopyrithiamin	Growth of pea roots in pure culture	thymine		Growth is inhibited by the antimetabolite.	Schopfer et al, 1951
36	succinyl-sulfathiazole	Growth of intact roots of Lupinus albus			Root growth was stimulated at 100 p.p.m.	Macht, 1945
37	sulfadiazine	ditto			Less inhibitory than sulfanilamide at 100 p.p.m. Root growth was stimulated in a dilute solution.	ditto
38	sulfaguanidine	ditto			Root growth was stimulated at 100 p.p.m.	ditto
39	ditto	Growth of Lemna minor			10^{-2} M was the lowest concentration which inhibited growth.	Fromm and O'Donnell, 1955
40	sulfanilamide	Growth of cress (Lepidium sativum L.)			At a concentration of 1/1000 inhibited growth.	Trefouel et al, 1935
41	ditto	i)Tomato cuttings ii)Germination of seeds			Higher concentrations of sulfanilamide are inhibitory to these higher plant growth systems but lower concentrations produce a stimulatory or hormonal effect. (According to Audus and Quastel (1948), this paper is hard to assess due to lack of experimental details with regard to controls, etc)	Grace, 1938
42	ditto	Growth of isolated tomato roots in pure culture	PABA		At 30 mg. per litre sulfanilamide the roots grew only 15 - 21% of the controls. PABA added to the above at 1 mg. per litre caused a growth increase of 3.3 to 4.5 times that of those in the sulfanilamide alone.	Bonner, 1942

TABLE II (CONTINUED)

43	ditto	i)Growth of Lupinus albus in physiological solutions ii)excised Lupinus albus roots in culture media			Less than 5 p.p.m. sulfanilamide stimulated growth where 40 p.p.m. or over inhibited growth. A mixture of sulfanilamide and PABA inhibited growth more than either one of the compounds alone. This result was true in both of the test systems and is in direct opposition to the results obtained by previous workers.	Macht and Kehoe, 1943
44	ditto	Length increase of Pisum sativum roots grown in agar under sterile conditions	PABA	competitive	Sulfanilamide at 300 p.p.m. caused complete inhibition of root length increase. The data obtained fit in with the Woods-Fildes (1940) theory of sulfonamide action.	Wielding, 1943
45	ditto	Germination of Oryza sativa L.	PABA	competitive	The results indicate that the antagonism of PABA is the same as in cultures of bacteria.	Ribeiro, 1944
46	ditto	Growth of intact roots of seedlings of Lupinus albus			Sulfanilamide inhibited but low concentrations (100 p.p.m.) promoted growth. Sulfanilamide : PABA :1:1 (in different concentrations) caused an inhibition. These data are unlike others reported by workers on bacteria.	Macht, 1945
47	ditto	Division of plantlets of Lemna minor and formation of new fronds			Sulfanilamide at 5×10^{-4} M inhibits the division of the plants. At 10^{-3} M it inhibits the formation of new fronds. PABA at 10^{-4} M counteracts these inhibitions in part.	Fromm and O'Donnell, 1953
48	ditto	Growth rate of flax seedlings			30% inhibition occurs at 1.04×10^{-4} M.	Moore, 1953a
49	ditto	ditto	i)PABA ii)pteroic acid iii)p-amino benzoylglutamic acid iv)PGA	competitive competitive competitive	50% inhibition occurs at 4.4 log molar units. No reversal of the inhibition was obtained by the purines tested. This is unusual considering the reports of other workers that they are secondary antagonists. Moore suggests that since these previous results were based on growth extent per unit time and not on growth rate they must be considered as of uncertain significance.	Moore, 1953b

TABLE II (CONTINUED)

50	ditto	Growth of isolated tomato roots in sterile culture	PABA PGA methionine	competitive competitive	Growth was measured in terms of increases in 1)length of the main axis, 2)in lateral root number produced per unit length of the main axis and 3)the total length of the ten basal laterals. In one experiment the inhibition due to an excess of PGA was relieved by a concentration of sulfanilamide which was itself inhibitory. Sulfanilamide stimulated growth at low concentrations. Methionine was slightly antagonistic toward sulfanilamide. A difference between the effect of the same concentration of sulfanilamide, or concentrations of sulfanilamide plus antagonist, on growth of the main axis and lateral is of some interest with regard to the morphogenesis of the root.	Boll, 1955
51	sulfanilamido-thiazole	Growth rate of pea roots cultured	PABA guanine adenylic acid ribonucleic acid		The antimetabolite at 10^{-5} M reduces the growth rate by about 70%.	Schopfer and Anker, 1949
52	ditto	Growth of Pisum sativum roots in pure culture	PABA		At a concentration of 1:24 x 10 M of the antimetabolite to PABA there is a 24% increase over the control in laterals produced.	Anker, 1951
53	sulfapyridine	Growth of intact roots of Lupinus albus seedlings			Less inhibitory than sulfanilamide at 100 p.p.m. Root growth is stimulated in a dilute solution.	Macht, 1945
54	ditto	Growth of isolated tomato roots in sterile culture	PABA		The antimetabolite at 30 mg. per litre caused the roots to grow only 15 - 21% of the control. When 1 mg. per litre of PABA was added to the above the growth was increased by 3.3 to 4.5 times.	Bonner, 1942
55	sulfathiazole	ditto	PABA	competitive	50% inhibition occured at 2 mg. per litre. The antimetabolite at 30 mg. per litre caused the roots to grow only 15 to 21% of the control. When 1 mg. per litre of PABA was added to the above the growth was increased by 3.3 to 4.5 times.	ditto
56	ditto	Growth of intact roots of Lupinus albus seedlings			Less inhibitory than sulfanilamide at 100 p.p.m. Root growth was stimulated in a dilute solution.	Macht, 1945
57	tauramide	Lemna minor growth			8×10^{-2} M killed the plantlets. 8×10^{-3} M minimum concentration which inhibited growth.	Fromm and O'Donnell, 1955

TABLE III ANTIMETABOLITES TO NUCLEIC ACID DERIVATIVES (BIOCHEMICAL DATA)

TABLE III ANTIMETABOLITES TO NUCLEIC ACID DERIVATIVES (BIOCHEMICAL DATA)

#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCE
1	aza-adenine (AA)	Spirodela oligorrhiza (Kurtz) Hegelm in sterile culture	adenine hypoxanthine		AA inhibits anthocyanin formation at 10^{-5} M. The reversal compounds reverse this inhibition at 10 times the AA concentration. **The evidence suggests that there are 2 stages in anthocyanin formation: (I) a light reaction with a Cu-enzyme involved and for the synthesis of a nucleotide or one or more pyrimidine or purine compounds; and (II) a dark reaction which utilizes the products of this light reaction for formation of anthocyanin.	Thimann and Radner, 1955b
2	aza-guanine (AG)	Various virus infections on various plants (See Table IV)			The view that AG may act by replacing guanine in virus synthesis is proposed here. Of the three tri-azolopyrimidines tested AG was the only one which was effective in inhibiting virus spread or reproduction. It was also the only one with the same substituted groups in the same position as guanine.	Matthews, 1951
3	ditto	Lucerne mosaic virus infected leaves of Nicotiana glutinosa Linn.			A yellowing of the leaves occured. Pigment formation was interfered with.	Matthews, 1953 a
4	ditto	Tobacco mosaic virus in tobacco leaves			The results show that AG is incorporated into the nucleic acid of tobacco mosaic virus and also that at least some plant viruses can use free guanine for synthesis. It is thought that the incorporation of AG into the virus nucleic acid renders the particle "sterile".	Matthews, 1953b
5	ditto	Growth of decotylised seedlings of Pisum sp. grown in sterile agar media	adenine (A) hypoxanthine (H)		The nucleic acid content of the root and shoot decreased with increasing concentrations of AG. At 0.01 uM AG inhibited nucleic acid production. A or H (purine: AZ ratio of 30) with AG permitted almost normal nucleic acid production.	Fries, 1954
6	ditto	Tobacco mosaic virus in tobacco leaves			It was shown that 3 - 4% of the guanine in the virus nucleic acid was replaced by AG.	Matthews, 1954
7	benzimidazole	Spirodela oligorrhiza anthocyanin formation			Anthocyanin formation was inhibited to 96% of the control at 3×10^{-4} M (slight inhibition only). See** #1 above.	Thimann and Radner, 1955b

TABLE III (CONTINUED)

8	2-6-diamino-purine (2-6-DAP)	ditto	hypoxanthine (H)		AG inhibited pigment formation at 2×10^{-4} M (very high). Reversal studies were difficult to carry out because very high concentrations of purines were needed and at these high concentrations most purines are themselves inhibitory and guanine in particular, is insoluable. H reversed 4% of the inhibition. (See ** #1 above).	ditto
9	thiouracil (TU)	Tobacco mosaic virus biosynthesis in isolated sterile discs which are in nutrient media and illuminated	uracil (U)		The rate of virus synthesis is completely inhibited at 4×10^{-5} M TU. An excess of U partially reverses the inhibition. TU appears to have no effect on the properties of the virus molecule.	Commoner and Mercer, 1952
10	ditto	Spirodela oligorrhiza grown in water non-nutrient media and in bright light			TU at 1.3×10^{-5} M caused 25% inhibition of anthocyanin formation. The importance of the sulfhydryl and sulfone groupings were assessed by studying the effects of various "s" containing compounds on anthocyanin formation. (See Table V.)	Thimann and Radner, 1955a
11	ditto	ditto	uracil (U) copper ions (C) thymine (T) adenine (A) hypoxanthine (H)		TU inhibits anthocyanin formation 50% by 2×10^{-5} M in the light. TU inhibits the pigment formation by interfering with copper containing enzymes. Reversal by U, C and T is complete, but by A and H only partial, when the M concentration is 10 times that of TU. TU in dark at 2×10^{-5} M (see above) is stimulatory to pigment formation. See ** #1 above).	Thimann and Radner, 1955b
12	quinine (Q)	ditto	adenine (A) hypoxanthine (H)		Anthocyanin formation is inhibited by Q at 3×10^{-5} M. Purines partially reversed the inhibition at 6×10^{-4} M.	Thimann and Radner, 1955b

TABLE IV ANTIMETABOLITES TO NUCLEIC ACID DERIVATIVES (PHYSIOLOGICAL DATA)

TABLE IV ANTIMETABOLITES OF NUCLEIC ACID DERIVATIVES (PHYSIOLOGY DATA)

#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCES
1	7-amino-1-v-triazolo (D) pyrimidine = (Triazolo analogue of adenine)	Growth of host plants and a number of viruses which infected them			The analogue affected the viruses slightly but severely damaged the host plants.	Matthews, 1953a
2	8-azaguanine = (guanazolo) (AG)	Growth of virus tumor on Rumex acetosa L.	guanine (G)		AG inhibited the tumor growth at all levels as low as 1 p.p.m. Therefore, the tumor tissue is very sensitive to G. A ratio of 100 or 200 G to 1 AG was necessary to reverse the inhibition.	Nickell.et al, 1950
3	ditto	(a)Stem fragments of garden Chrysanthemum (Var. Golden Treasure) inoculated with crown gall bacterium.			Growth of the resulting tumor tissue was inhibited by AG.	deRopp, 1950
		(b)Bacteria free crown gall tumor of sunflower in aseptic culture			AG inhibited the growth of the tissue at 1 mg. per litre.	
		(c)Excised tomato roots in aseptic culture			ditto	
		(d)Excised sunflower embryos in aseptic culture			ditto	
					In a to d above no specific effect was exerted on healthy as opposed to tumor tissue. It appears that AG acts by interfering with cell division.	
4	ditto	Lesion production and systemic spread of (a)Lucerne mosaic virus on Nicotiana tobocum. var. White Burley			(a)Lesions were reduced by the presence of AG.	Matthews, 1951
		(b)Lucerne mosaic virus on N. glutinosa L.	adenine guanine hypoxanthine		(b) " " " " " " " " . AG caused a transient blotchy yellowing in the host. This host effect was not reversed by the reversal compounds.	

TABLE IV (CONTINUED)

		(c)Cucumber mosaic virus on cucumber (short prickly variety)			(c)AG delayed the systemic infection of the leaves.	Matthews, 1951
		(d)Pea mosaic virus in peas			(d)ditto	
		(e)Tomato spotted wilt virus on tomato			(e)The virus was not affected but the host was. A transient blotchy yellowing occured in host tissue.	
		(f)Potato virus x on tobacco leaves			(f)Systemic spread of the virus was delayed.	
		(g)Acuba strain of tobacco mosaic virus on tomato leaves			(g)ditto	
5	ditto	Growth of stem fragments of garden chrysanthemum var. Golden Treasure infected with crown gall bacterium			Growth of the stem fragments was inhibited by AG. Guanine did not reverse this inhibition.	deRopp, 1951
6	ditto	Growth of Stichococcus subtilis (Kutz) Klecher, in sterile culture	guanine (G)		The mechanism of growth inhibition of this alga by AG and its reversal by G. appears to parallel that found in other plant and animal systems which have been tested.	Arnow et al, 1952
7	ditto	(a)Lucerne mosaic virus in Nicotiana glutinosa	adenine (A) guanine (G) hypoxanthine (H)		(a)AG reduced the number of local lesions and delayed or inhibited systemic spread of the virus. AG above 0.005 M caused host damage i.e. yellowing and distortion of younger leaves with general stunting if treatments were prolonged.	Matthews, 1953a
		(b)Spotted wilt virus in tomato			(b)AG had slight or no effects on lesion formation or spread.	
		(c)Potato virus x in potato			(c)ditto	
		(d)Potato virus y in potato			(d)ditto	
		(e)Potato mosaic virus in peas			(e)ditto	
		(f)Pea mosaic virus in peas			(f)ditto	
		(g)Cucumber mosaic virus on Short, Prickly Cucumber leaves			(g)When 0.01 M AG was applied mechanically it delayed or prevented the systemic movement of the virus in the leaves but it had no effect when it was watered on the soil.	

TABLE IV (CONTINUED)

8	ditto	Tobacco mosaic virus in tobacco leaf discs			10^{-3} M AG caused 60% inhibition of virus multiplication.	Mercer et al, 1953
9	ditto	Growth of excised Pisum roots cultured in liquid media	guanine		AG at 0.01 - 10 µM was tested on root growth. Results were based on mean total length (and dry weight) of the total root system. Roots were readily branched so that the main root was difficult to distinguish from the laterals. AG at 0.1 µM reduced growth considerably and at 0.3 µM suppressed it almost completely. With a guanine: AZ ratio of 30 a partial prevention of this inhibition was obtained.	Fries, 1954
10	ditto	Root growth and morphology of cotyledonless Pisum seedlings grown in sterile agar media	guanine (G) adenine (A) hypoxanthine (H)		AG at 0.01 - 10 µM was tested on the seedlings. A ten times stronger solution of AG was necessary to produce the same inhibition of root growth here as occured above (excised Pisum roots). AG at 0.5 µM reduced the growth rate to less than 50% of the control. With 10 µM of AG suppression of growth was almost complete. A rather high purine: AG ratio of 60 was necessary for a complete removal of the growth inhibition. (Purines were in a concentration of 0.3 µM and AG at 0.005 µM). The purines produced quite idfferent morphological effects: (a)G did not prevent the usual complete inhibition of lateral roots by AG. (b)A and H almost completely restored the growth rate of the main root and even increased the number and growth rate of the lateral roots compared to the control. (c)A or H in purine/AG ratio of 30 permitted only partial restoration of shoot and root elongation and dry matter increase.	ditto
11	ditto	Nicotiana glutinosa leaves infected with Lucerne mosaic virus	adenine (A) guanine (G)		AG reduced local lesions, delayed or inhibited systemic spread of the virus and caused negligible plant damage.	Nickell, 1955
12	ditto	Some plant cells			AG had an antimitotic effect.	Deysson et al, 1956
13	ditto	Decotylized Pisum seedlings in synthetic media	adenine hypoxanthine		AG depressed the growth rate of the seedlings. This was partially restored by the reversal compounds at 30 times the concentration of AG.	Fries, 1954
14	ditto	Datura stramonium L crown gall tumors			AG at 1 gm. per litre stopped tumor growth. AG had no effect on the host. Guanine did not antagonize the inhibition of growth caused by AG.	Manil and Fourneau, 1956

TABLE IV (CONTINUED)

15	Benzimidazole (BZ)	Growth of sub-apical sections of etiolated Alaska pea epicotyls	adenine (A) guanine (G) adenosine (Ad.) guanylic acid (Ga.) adenylic acid (Aa)		BZ stimulates water uptake but inhibits cell elongation while causing an extension of the cortical cells in a transverse direction. The reversal compounds partially reverse cell elongation inhibitions which suggests that purine containing compounds such as nucleic acids are implicated in the auxin controlled elongation of plant cell walls.	Galston et al, 1953
16	ditto	Growth of Lemna minor in aseptic culture			At 3.4×10^{-3} M - BZ inhibits root elongation and increases frond area by 65 - 75% due to cell enlargement. Auxin will modify the plants in the same way but death follows. BZ modified plants do not die.	Hillman, 1955
17	ditto	Growth rate of Linum usitatissimum L			50% inhibition of growth rate occured at approximately 3.6 Log molar units of BZ. This was true whether the growth rate was measured in terms of amount of water uptake or in terms of all-over seedling length increase.	Moore, 1953a
18	ditto	Change in morphology of Lemna minor in aseptic culture			BZ modifies plant form. Root elongation is completely inhibited and a 65 - 70% increase in frond area occurs due to cell elongation. These modified plants exhibit a higher sensitivity to auxins and a lower sensitivity to the anti-auxin 2,4,6 - T than normal plants do.	Hillman, 1955
19	ditto	Growth of primary roots of Vicia faba L seedlings Mitotic activity of above studied	manganous ions calcium ions		BZ inhibited the elongation of the primary roots, inhibited mitotic activity in the root meristem and induced the formation of black pigments in the regions of the root above the meristem. Calcium ions prevented the inhibition of elongation by BZ. Maganous ions prevented largely the growth inhibitions produced by BZ. The data indicate that BZ is not acting as a strict anti-metabolite as was previously suggested. It is possible that the mode of action here is via a chelating mechanism.	McCorquodale and Duncan, 1957
20	2,6-diamino-purine (2-6-DAP)	(a)Systemic development of Lucerne mosaic virus on tobacco leaves (Nicotiana tabacum var. White Burley) (b)Ring spot strain of virus on Tobacco leaves (?) (c)Growth of the host plants of above			(a)2-6-DAP delayed the systemic development of the virus. (b)ditto (c)When the plants were treated with 2-6-DAP after they were fully infected with a virus their growth was stunted.	Matthews, 1951

TABLE IV (CONTINUED)

21	ditto	Tobacco mosaic virus synthesized in ?			2-6-DAP prevented virus synthesis.	Ryzhkor et al, 1952
22	ditto	Tobacco mosaic virus synthesized in tobacco leaf discs which were in nutrient culture			10^{-4} M 2-6-DAP inhibited virus multiplication by 80%.	Mercer et al, 1953
23	ditto	(a)Cell division in tobacco stem segments grown in sterile culture			2-6-DAP inhibited cell division. G and H have only a slight reversal effect.	Miller, 1953
		(b)Cell division in habituated tobacco callus (4 years in culture)	adenine (A) guanine (G) hypoxanthine (H)		2-6-DAP inhibited cell division.	
		(c)Formation of buds on callus from internal phloem of tobacco	A G H		2-6-DAP inhibited bud formation. G and H have only a slight reversal effect.	
		(d)Enlargement of cells in tobacco stem pith cultured in vitro	A		2-6-DAP inhibited cell enlargement.	
		(e)Etiolated pea (Alaska #323) stem segments grown in liquid media	A adenosine adenylic acid		50 mg. per litre of 2-6-DAP inhibited cell elongation 40%. The reversal compounds gave only partial reversal. 2-6-DAP also inhibited $CoCl_2$ induced elongation.	
		(f)Cell enlargement in *Avena* coleoptile segments floated in solutions			2-6-DAP reduced cell enlargement.	
24	ditto	*Lemna minor* growth in sterile media	A		50% inhibition by 2-6-DAP occured at approximately 0.1 p.p.m. The ratio at which no inhibition occurs is 20A to one 2-6-DAP. Higher levels were not tested.	Nickell, 1955

TABLE IV (CONTINUED)

25	ditto	Sterile cultures of: (a)sweet clover callus (b)Pole bean callus (c)Sunflower crown gall callus (d)Tobacco crown gall callus (e)Sorrel virus tumor callus	adenine (A) A A A		Callus tissues were least inhibited by 2-6-DAP. Crown gall tissues were inhibited more by 2-6-DAP. Virus tumor tissue was the most inhibited by 2-6-DAP. The author suggests "a progressive degree of pathological conditions are represented here."	ditto
26	ditto	Various plant cells			2-6-DAP had an antimitotic effect.	Deysson et al, 1956
27	7-hydroxy-1-v-triazolo (D) pyrimidine = (Triazolo analogue of hypoxanthine)	Various plants infected by various viruses			This analogue was less effective than azaguanine in reducing the number of local lesions produced in tobacco (Nicotiana tobacum var. White Burley and N. glutinosa) but it was more effective in reducing the number of local lesions produced in Phaseolus vulgaris L var. Sidney Wonder. The analogue caused no host damage.	Matthews, 1953 a
28	6-Mercaptopurine (6-MP)	Growth of Rumex virus tissue in vitro			50% inhibition of growth occured at approximately 10 p.p.m. 6-Mp.	Nickell et al, 1950
29	ditto	Numerous plant cells			6-Mp had an antimitotic effect.	Deysson et al, 1956
30	ditto	Lemna minor tissue			Growth was stimulated by 6-Mp at 1 - 10 p.p.m.	Nickell, 1956
31	4-methyl-2-thiourocil	Growth of oats, mustard, cress, and buckwheat plantlets			Growth of these plants was inhibited by the analogue but not as strongly as by T.U. (see below).	Ber, 1949
32	5-methyl-2-thiouracil	ditto			ditto	ditto
33	6-methyl-2-thiouracil	ditto			ditto	ditto

TABLE IV (CONTINUED)

34	thiouracil (TU)	Growth of: (a)<u>Pisum</u> plantlets	Uracil (U)		(a)TU inhibits the growth of shoots, side roots and root hairs. It promotes the development of the main root and promotes the growth of lateral buds forming bushy plants.	ditto
		(b)Mustard plants	U		(b) and (c)TU inhibits the growth of shoots, side roots and root hairs but it promotes the development of the primary roots.	
		(c)Cress plants	U			
35	ditto	Cress seed germinating on single layers of lint in petri dishes	U		TU inhibits germination. U reverses this inhibition at 1:20 TU to U.	Trotter, 1949
36	ditto	Tobacco mosaic virus grown on leaf disc tissue in nutrient media and illuminated	U		Virus multiplication is inhibited by low concentrations of TU. U partially reverses this inhibition. No virus is formed if the TU concentration is 4×10^{-5} M or greater.	Commoner and Mercer, 1952
37	ditto	Plants	U			Ber, 1953
38	ditto	Leaves of <u>Nicotiana</u> sp. infected with Lucerne mosaic virus			TU caused severe host damage but was ineffective on the virus.	Matthews, 1953a
39	ditto	Leaf discs infected with tobacco mosaic virus			At 10^{-4} M, TU inhibited the multiplication of the virus by 90 - 100%.	Mercer et al, 1953
40	ditto	Growth of <u>Linum usitatissimum</u> seedlings			30% inhibition of the growth rate occured at a 1.32×10^{-4} M.	Moore, 1953b
41	ditto	(a)Tobacco mosaic virus	U		Multiplication of all viruses was inhibited by TU. Multiplication of (g) and (h) was not inhibited. Plant growth was impared by TU but this inhibition was not reversed by U.	Bawden and Kassanis, 1954
		(b)Tobacco necrosis virus	U			
		(c)Potato virus y	U			
		(d)Tomato acuba mosaic virus				
		(e)The Rothamsted culture of tobacco mosaic virus				

TABLE IV (CONTINUED)

		(f)Henbane mosaic virus in Nicotina tobacum L var. White Burley (g)Tobacco necrosis virus in French bean (h)Mottle virus in Vicia faba				
42	ditto	Tobacco leaf discs innoculated with tobacco mosaic virus			TU inhibited the multiplication of the virus. An absorption of 0.013 mg. per 24 hours by 16 - 14 mm. discs was the lowest absorption which produced maximum virus inhibition. This is the only data containing actual measures of amounts of the antimetabolite which are absorbed. For this reason it is an outstanding paper.	Nichols, 1954
43	ditto	Crown gall of Datura sp. in sterile culture	Uracil		TU inhibited gall growth at 1 gm. per litre.	Manil and Fourneau, 1955

TABLE V ANTIMETABOLITES TO AMINO ACIDS (BIOCHEMICAL DATA)

TABLE V ANTIMETABOLITES TO AMINO ACIDS (BIOCHEMICAL DATA)

#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCE
1	asparagine	Growth of Capsella bursa-pastoris Medic embryos	glutamine		Asparaginemay block a specific enzyme to its natural metabolite - glutamine.	Rijven, 1955
2	azaserine (AS)	Growth of photosynthesising cells of: (a)Scenedesmus sp. (b)Chlorella sp.	(a)glutamine (b)none		Glutamine partially reverses the inhibition. The data indicates that one of the major sites of glutamine action is in reactions involving transamination. A more widespread interference by AS, rather than just an interference with purine synthesis, is indicated. Six interference areas are suggested.	Barker et al, 1956
3	canavanine (C)	Growth of sections of Avena coleoptiles	arginine lysine glutamic acid IAA	competitive	Depending upon the IAA content, 50% inhibition by C occurs at 3 - 10 mg. per litre. Arginine seems to play a role other than that of supplying organic nitrogen to the coleoptile. It is possible that it supplies a certain special protein which is not produced in quantity enough to be detected by protein qualitative and quantitative tests. It is suggested (1) that arginine metabolism may be associated with the function of IAA in promoting growth, and (2) that glutamic acid is a precursor in arginine synthesis as has been shown in microorganisims.	Bonner, 1949a
4	ditto	Respiration in growing sections of Avena sp. var. Siegeshafer	arginine		C inhibits the increase in respiration due to IAA but does not inhibit the increase due to adenylic acid. It is concluded therefore that the reactions involved in Avena section growth and respiration are the following: respiration adenylic acid arginine step IAA step.	Geo. Rieveschl, Park, Davis and Co., 1955
5	ethionine (E)	Growth of isolated tomato roots in sterile culture	(a)methionine (b)homocysteine (c)ethanolamine		The antagonisims of a,b, and c toward the inhibition of growth by E indicates that they are related metabolically in the isolated tomato root. Such a relationship has been well established in microorganisims and animals.	Boll, 1954d
6	ditto	Anthocyanin formation in Spirodela oligorrhiza grown in non nutrient water media and in bright light			Methionine and ethionine inhibit the pigment formation - not via their sulphur atom but by some property of the molecule as a whole. Ethionine inhibits ten times as much as methionine. Ethionine does not inhibit the production of the small amount of pigment which is formed in the dark. If E is preilluminated with the plants before they are placed in the dark then E does inhibit the normal formation of anthocyanin in the dark. It is deduced that E must inhibit a light reaction whose product is converted to anthocyanin in the dark.	Thimann &Radner, 1955a

TABLE V (CONTINUED)

7	ditto	ditto			The data suggest that the inhibition of anthocyanin formation by methionine and E is due to their structural similarity to pyrimidines i.e. thymine.	Thimann and Radner, 1955b
8	ditto	Elongation of Avena coleoptile sections	L-methionine	competitive	50% inhibition of growth occurs at 10 mg. per litre E. The data indicate that these tissues can convert homocysteine to methionine in the presence of ethionine. This is true of other organisims. It would appear that E is inhibiting the use of methionine rather than its synthesis.	Schrank, 1956
9	methionine sulfoxide (MS)	Anthocyanin formation in Spirodela oligorrhiza grown in water non-nutrient media			MS inhibits pigment formation 49% at three times 10^{-5} M. Since both MS and MSI are less effective than methionine in inhibiting the pigment formation the inhibitory action of methionine cannot be due to its conversion to either of them.	Thimann and Radner, 1955a
10	methionine sulfoximine (MSI)	ditto			MSI inhibits pigment formation 43% at 10^{-5} M.	ditto
11	ditto	(a)Growth of Chlorella vulgaris in pure culture (b)Lesion production in healthy tobacco leaves	methionine	competitive		Wooley, 1955
12	norleucine	Anthocyanin formation in Spirodela oligorrhiza			It is suggested that norleucine is structurally enough like thymine to replace it partially in the nucleic acid or purine or pyrimidine reactions necessary for anthocyanin formation.	Thimann and Radner, 1955b
13	Pseudomonas tabaci toxin (amino acid)	(a)Growth of Chlorella vulgaris in pure culture (b)Lesion production in healthy tobacco leaves	methionine	competitive	This is an example of a naturally occuring antimetabolite. The fact that the toxin is an amino acid while most bacterial toxins are proteins is of interest. As plants do not have the blood stream transportation system of animals it would appear that the parasite has been specially provided with a small, readily diffusible toxin rather than an outwardly large, slow-travelling protein molecule.	Wooley, 1955
14	L-tryptophane	Root growth of cress seedlings			Root growth inhibition is detected at more than 10 p.p.m. and 50% inhibition occurs at 100 p.p.m. with 80% inhibition at 1000 p.p.m. tryptophane. It is suggested that tryptophane may effect the inhibition of root growth by competitive interference in the normal transaminase systems involving α-alanine and l-glutamic acid.	Audus and Quastel, 1947

TABLE VI ANTIMETABOLITES TO AMINO ACIDS (PHYSIOLOGICAL DATA)

TABLE VI ANTIMETABOLITES TO AMINO ACIDS (PHYSIOLOGICAL DATA)

#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCES
1	azaserine O-diazoacetyl-L-serine (AS)	(a)Primary root elongation of cucumber (Var. Early Fortune) seedlings (b)Growth of barley roots var. Moore in aerated water culture (c)Root elongation of germinating barley seedlings (d)Root elongation of germinating flax seedlings (e)Bean shoot growth			(a)50% inhibition of root development by AS occured at 1.5×10^{-5} M. At 2×10^{-4} M and 5×10^{-4} M root development was completely suppressed. (b)50% inhibition of roots occured at 1.3×10^{-5} M (2.25 mg. per litre). Root growth was based on dry weight measure. (c)50% inhibition occured at 2.5×10^{-5} M AS. (d)50% inhibition occured at 4×10^{-6} M AS (0.7 mg. per litre). (e)Unaffected up to 100 γ per litre when AS was applied to the bases of intact leaves or to detached leaf bases.	Norman, 1955
2	ditto	Several plant systems inhibited				Geo. Rievesdel, Paper #3
3	ditto	High potency for inhibiting root growth				Plant Nutrition Laboratory of the University of Michigan, Paper No. 2
4	ditto	Chromosome studies and root elongation of Allium cepa L.			AS caused chromosome fragmentation. AS inhibited root elongation at 1.5×10^{-8} M by inhibiting cell division.	Truhaut and Deysson, 1956
5	ditto	The germination of Triticum vulgare Vill.			AS inhibited either partially or completely the germination of the wheat. Serine does not antagonize the inhibition by AS. Complete inhibition of germination occurs at 2×10^{-5} M AS.	ditto
6	ditto	Growth of Chlorella pyrenoidosa Chick			AS at 0.01 M greatly inhibited the growth of the alga. The degree of inhibition varied with the pH of the media.	Tomisek et al, 1957

TABLE VI (CONTINUED)

7	Canavanine H_2N-C(:NH)-NH-O-CH_2-CH_2-CH(NH_2)-COOH (C)	Growth of *Avena* coleoptile sections	(i)arginine (ii)lysine (iii)glutamic acid (iv)IAA	competitive	50% inhibition of section elongation occurs at 3-10 mg. per litre. C is dependent upon the content of IAA. (ii)-(iv) reverse the inhibition of growth by C only partially. It would appear that (i) plays a role other than that of supplying organic Nitrogen to the coleoptile. It is suggested that the metabolism of (i) may be associated with the function of IAA in promoting growth here.	Bonner, 1949a
8	ditto	Growth of excised maize embroys cultured in vitro (2 hybrids used)	arginine citrulline ornithine glutamic acid lysine methionine	competitive	It was concluded that different genetic lines of maize seem to show variations in their sensitivity to canavanine inhibition and to the reversal of this inhibition by other amino acids. Lysine was an effective antagonist with only one hybrid. Methionine is a possible antagonist.	Wright and Srb, 1950
9	ditto	Growth and morphology of decotylised *Pisum* seedlings in agar culture	arginine (A) citrulline (Ci) ornithine (O)		The influence of 0.003 - 1.0 uM of C on shoot and root length and dry weight increase was observed. At low concentrations (0.3 uM) C reduced the number and growth rate of the lateral roots as well as the increase in dry matter of the whole seedling. At 10 µM, and higher concentrations the elongation of the shoot and root was affected. This inhibition was not completely prevented by A. A completely compensated for the decrease in dry matter due to C but affected less the reduction in root growth due to C. Every concentration of A tested (0.1 - 0.3 µM) removed some inhibition but not completely. (This was found to be true with pea embryos as well). Ci and O, two A precursors were as active as A in removing the inhibition by C. Lysine, methionine or glutamic acid did not remove C inhibition. It is suggested that a closer structural similarity of the antagonist to the inhibitor is necessary in this case than in others reported.	Fries, 1954
10	ditto	Growth and respiration in *Avena* seedlings var. Siegeshafer	arginine		The increase in respiration due to IAA is inhibited by C but not the increase due to adenylic acid. On the basis of the use of the inhibitors including C, it is concluded that the reactions here considered are involved in *Avena* section growth and reproduction in the following order: Respiration Adenylic acid Arginine IAA step.	Norman, 1955

TABLE VI (CONTINUED)

11	ditto	Growth of eight species of Green Algae inhibited (a)Chlorella vulgaris (Columbia strain) (b)Chlorella pyrenoidosa (c)Selenastrium minutum	L-lysine L-arginine DL-lysine L-arginine	competitive competitive	Growth of eight species was inhibited but only three were mentioned by name. (a) was inhibited 50% by 2.2 p.p.m. C. A higher concentration of C was needed to inhibit the growth of (b) due to the cells being less permeable to C than the other species tested. DL lysine is one half as active an antagonist as L-lysine - particularly at lower concentrations of C.	Walker, 1955
12	ditto	Growth of Lemna minor	arginine (A) citrulline (Ci) ornithine (O)		25% inhibition by C occurs at 1 p.p.m. and 75% at 5 p.p.m. A will reverse the inhibition caused by C at 5, 10, 20 and 50 p.p.m. if the ratio of A to C is 5 to 1. Above 50 p.p.m. C the inhibition could not be reversed by A. A+Ci overcame the inhibition by C and even caused a stimulation in growth above that of the control. O did not completely overcome the inhibition of growth by C.	Nickell, 1956
13	DL ethionine "E"	Growth of excised tomato roots in sterile culture	methionine (M) homocysteine (He) homocystine (Hi) ethanolamine (Eo)		Choline and glycine-betaine were ineffective in reversing the inhibition of growth caused by E. E and various ratios of M (or H) to E also caused some significant changes in the morphology of the roots.	Boll, 1954d
14	ditto	Growth and change in morphology of isolated tomato roots in sterile culture	L-methionine (M) DL-homocystine (Hi) ethanolamine (Eo)		E at a concentration below 0.2 standard (.2 x 60 gamma per litre) caused a marked increase in growth in length of the main axis with a corresponding increase in the number of laterals per root. In the absence of niacin higher concentrations of E caused an inhibition of growth of the main axis and the number of laterals per root. M reversed this inhibitory effect. In the presence of niacin two morphological changes were induced by E. (a) The lowest concentration of E tested caused a marked stimulation in growth of laterals. The main axis growth was inhibited at lower concentrations of E than those which inhibited the growth of the lateral roots. M reversed these inhibitions. (b) The frequency of the laterals per unit length was greater than the controls when an appropriate ratio of E to M was present in the media. The data suggests that the metabolism of M is involved with the hormonal mechanism involved with the growth of these isolated roots.	Boll, unpublished

TABLE VI (CONTINUED)

15	L-ethionine (L-E) DL-ethionine (DL-E)	Elongation of Avena coleoptile sections ditto	L-methionine DL-homocysteine thio-lactone-HCL	competitive	E at 10 mg. per litre inhibits the elongation 50%. The inhibition index for E to M 2:3. The data indicates that L-E interferes with the utilization of M for syntheses which are required for coleoptile elongation. DL-E inhibition is one-half as effective as the inhibition by L-E.	Schrank, 1956
16	L-ethionine	Curvature of isolated Avena coleoptiles with intact apexes	L-methionine		This reversal of E inhibition by M is only true on short term experiments. After 24 hours M itself becomes inhibitory.	ditto
17	homoarginine	Growth of Chlorella vulgaris (Columbia strain)	L-lysine L-arginine DL-lysine		H inhibits the growth of the alga more so than canavanine. DL-lysine is one-half as strong a reversing compound as L-lysine.	Walker, 1955
18	hydroxyproline (Hp)	Growth and lateral root production in decotylised Pisum seedlings				Fries, 1951
19	ditto	Decotylised Pisum seedling growth in sterile agar culture	proline (P) methionine (M)		10 - 300 µM of Hp were tested on the root growth. 1 µ mole was the smallest amount of Hp to cause maximum inhibition. Equimolar amounts of amino acids were tested and none removed the inhibition completely. P completely removed the inhibition of dry weight increase and removed 50% of the inhibition of elongation (particularly root elongation). M relieved the shoot inhibition in particular. P and Hp in a ratio of 10 to 1 removed the inhibition of dry weight by Hp to the extent that the weight of root and shoot surpassed those of the control even though the length of the primary root and the number of laterals produced was still supressed. M and a small amount of Hp increased the favourable effect of Hp on the shoot inhibition but intensified the inhibition of lateral root development caused by Hp.	Fries, 1954
20	methioninesulfoximine	(a)Production of lesions in healthy leaves of tobacco (b)Growth of Chlorella vulgaris in pure culture	methionine			Wooley, 1955

TABLE VI (CONTINUED)

21	selenomethionine (SM)	Growth of Chlorella vulgaris	methionine	competitive	M reversed the toxicity of SM and also prevented its absorption depending upon the S/se ratio. SM induced a lag phase in the presence of sulphate. Cell division was inhibited but not growth in cell size or increase in dry weight. The cells which emerged from the lag phase were used as inoculum and were found to grow in the presence of SN without a lag phase.	Shrift, 1954 b
22	DL-tryptophane	Growth of flax seedlings			50% inhibition occured at 8.91×10^{-4} M of DL-tryptophane. The results here were in accordance with those made by Audus and Quastel (1947) on cress seedlings.	Moore, 1953 b
23	Pseudomonas tabaci toxin (amino acid)	Production of lesions in healthy tobacco leaves			The fact that the toxin is an amino acid while most bacterial toxins are proteins is of interest. As plants do not have the blood transportation system and possess thick cell walls (both of which are barriers to fast travel by large molecules) it would appear that this parasite has been specially supplied with a small molecular weight toxin to help counteract these plant barriers to systemic spread. The discovery is of significance when one considers the possibility that naturally occuring antimetabolites may prevent biological toxicity by excess quantities of naturally occuring metabolites.	Wooley, 1955
	ditto	Growth of Chlorella vulgaris in pure culture	methionine	competitive		

TABLE VII ANTAGONISIMS BETWEEN INORGANIC IONS

TABLE VII ANTAGONISIMS BETWEEN INORGANIC IONS

#	ANTIMETABOLITE	TEST SYSTEM	REVERSAL COMPOUND	TYPE OF INHIBITION	COMMENTS	REFERENCE
1	selenate	Growth of seedlings of Hard Federation wheat in nutrient media	sulfate	competitive	The competition occurs at the membrane where these ions are taken up. "It was Cameron (1880) who first proposed that selenium can be utilized instead of sulphur in the synthesis of organic compounds."	Hurd-Karrer, 1938
2	selenate	Green plants	sulfate	competitive		Trelease and Beath, 1949
3	selenium (Sc)	Chorella vulgaris growth rate	sulphur (S)	competitive	Sulphur prevented the absorption of selenium. Two interdependent processes were indicated: (1) competition between the analogues for sites at the cell membrane; and (2) competition within the cell for an enzyme system responsible for conversion of the metabolite. A competitive inhibition was indicated because at any one ratio of S to Sc regardless of the absolute level of each the growth response remained the same.	Shrift, 1954a

2.32 MAIN POINTS ARISING FROM THE TABULAR REVIEW

The papers reviewed in Tables I to VII show that, although studies involving the application of antimetabolites to plants are not very extensive as yet, a number of fields of research are beginning to apply antimetabolites as an aid to solving certain problems. The more interesting of these applications are mentioned in the following paragraphs.

2.321 THE SULFANILAMIDE QUESTION

In view of the work which has been done with microorganisms and animals it is not surprising to find, throughout the literature, references to considerable research into the effects of sulfonamides on plant systems. These researches indicate for the most part, that the mechanism of action of the sulfanilamides in plants is similar to that found to occur in microorganisms and animals (Table I, #12-15) and that the metabolic pathways in which PABA is involved are similar in plants, animals and microorganisms. In plants there has been some evidence which would tend to suggest that PABA, or some derivative, is involved in the biosynthesis of pyrimidines or their derivatives. Anker (Table I #7), has shown that, with isolated pea roots in sterile culture, inhibition by a sulfonamide is reversed by thymine, cytosine and uracil and Boll (Table I #19) has shown that inhibition of a strain of tomato roots by sulfanilamide can be overcome by thymine, cytidine, cytidylic acid and uridylic acid. In animals and microorganisms only thymine has been shown to reverse similar inhibitions. It would appear therefore that in this one respect the metabolism of PABA may differ in plants from that found in animals and microorganisms.

2.322 THE STUDY OF ANTHOCYANIN FORMATION

By the use of aza-adenine, benzimidazole, 2-6-diamino-purine and thiouracil (antimetabolites to nucleic acid derivatives) and of canavanine, methionine sulfoximine and norleucine (antimetabolites to amino acids) it has been shown (Tables III and V respectively) that there is evidence to support the view that there are two stages involved in anthocyanin formation. One is a light reaction involving a copper enzyme and results in the synthesis of a nucleotide or one or more pyrimidine or purine compounds. The second is a dark reaction which utilizes the products of this light reaction.

2.323 ANTAGONISMS BETWEEN INORGANIC IONS

Martin (1951), has suggested that in addition to antagonisms between organic metabolites, antagonisms between inorganic ions are likely to play an extremely important role in biological processes. Table VII provides evidence in favour of Martin's suggestion. It contains two examples of competitive inhibition by ions in plants.

2.324 STUDIES ON PLANT VIRUS INFECTIONS

Numerous experiments on the effect of nucleic acid antagonists on virus infections in plant tissues have shown some interesting results. 1) A difference in response of host and virus is indicated. Multiplication of the virus may be inhibited by the antimetabolite with (Table IV, #4b) or without (Table IV, #11) detrimental effects on the plant tissue. On the other hand only the host may be affected (Table IV, #4e). When the virus and the host tissue are inhibited by the antimetabolite, a reversal of the virus inhibition may be accomplished by applying the metabolite. A

reversal of the host inhibition does not always occur as well (Table IV, #41). In at least one case the virus is inhibited by a low, and the host plant by a higher, antimetabolite concentration (Table IV, #7). 2) Different viruses differ in their response to the antimetabolite. An example of this is the inhibition, by aza-guanine, of Lucerne mosaic virus on Nicotiana tabacum and Nicotiana glutinosa, and Acuba strain of tobacco mosaic virus on tomato leaves but not of tomato spotted wilt virus on tomato leaves (Table IV, #4). Of the viruses which were inhibited, only the inhibition of Lucerne mosaic virus on N. glutinosa was reversed by the metabolites adenine, guanine and hypoxanthine. 3) The mechanism of inhibition of virus multiplication by an antimetabolite appears to differ from one virus to another. In some cases, for example, the inhibition of tobacco mosaic virus by thiouracil (Table III, #9), the antimetabolite appears to prevent the incorporation of the metabolite into the virus particle. In other instances the antimetabolite itself is incorporated into the virus particle and renders the particle sterile. An example of this is the inhibition of tobacco mosaic virus by aza-guanine (Table III, #4 and #6). A practical use of antimetabolites in the treatment of plants infected by viruses is suggested by the above data. An antimetabolite which could inhibit virus multiplication without interfering with the nucleic acid relations of the host plant should be a valuable chemotherapeutic agent in the field of plant pathology.

2.325 THE PROBLEM OF NORMAL VERSUS PATHOLOGICAL CONDITIONS

In some studies with 2,6-diaminopurine (an antimetabolite to adenine) on healthy and pathological plant tissues (Nickell, 1955), it was observed that there was a difference in response to the antimetabolite by callus

tissues, crown gall tissues and virus tumor tissues (Table IV, #25). Nickell suggested that these tissues represent a progressive degree of pathologicalness in that the normal callus tissue was the least, the crown gall tissues more, and the virus tumor tissues the most inhibited by 2-6-diaminopurine. It is suggested that by observing the differences in response to different antimetabolites of normal versus pathological tissues the basic biochemical differences between the tissues could be determined and thus the biochemistry of the pathological condition could be defined. Once this has been done, it should be possible to devise a treatment for correcting the pathological condition.

2.326 NATURALLY OCCURRING ANTIMETABOLITES

Of particular interest in the literature is the report of the discovery of a naturally occurring antimetabolite, the Pseudomonas toxin, which is an amino acid (Table V, #13). This discovery (Wooley, 1955) is of great interest for two reasons. 1) The fact that this toxin is an amino acid of small molecular weight is worth noting since most bacterial toxins known to date are large protein molecules. It is suggested that some bacteria produce smaller, more easily diffusible toxin molecules which travel readily through the plant host which lacks the blood circulatory and lymph transportation systems found in animal hosts. This smaller molecule may also pass more easily through the thick cell walls of plants than would a molecule of a higher molecular weight such as a protein. 2) There are few examples in which antimetabolites of natural occurence have been discovered. It is known that the natural amino acids can act as antimetabolites (Table V, #3). Wooley's compound, however, is the only known natural antimetabolite which is not known to be a metabolite. This

discovery is of particular significance when one considers that it is possible that natural antimetabolites may control the rate of biochemical processes and hence growth. Wooley's discovery justifies a search for other natural antimetabolites which may be important in the regulation of growth and morphogenesis by acting as growth regulators.

2.327 MORPHOGENETIC EFFECTS OF ANTIMETABOLITES

Throughout the literature review there are numerous examples of antimetabolites affecting the morphology of plants or their isolated organs. Included among these are those of a sulfonamide on Allium cepa (Table II, #6) and of canavanine on decotylised Pisum seedlings (Table VI, #9). Of particular importance with regard to the subject of this thesis is the work by Boll which deals with the effects of ethionine on the growth of excised tomato roots in sterile culture (Table VI, #14). The next section will explain the method of culturing isolated tomato roots, the evidence for the presence of a hormonal, correlative factor within them, and the effects of ethionine upon the roots.

2.4 THE EFFECTS OF ETHIONINE ON ISOLATED TOMATO ROOTS

The excised root culture technique used is as follows. A sterile root is excised from a germinated seed, the coat of which has been sterilized prior to germinating it. The root is transferred to a sterile medium in which it grows and produces a number of lateral roots. The main axis so formed is cut into segments each having a number of lateral roots. When these segments are transferred to fresh medium the lateral roots grow and at an appropriate time the tops are excised and transferred again. In this way a clone is established and thus any number of growing tips are obtained.

Boll's experiments consisted of a series of different media inoculated with tips which, after growing for six days, were harvested and their growth was recorded.

The basal medium used was a modified White's medium (Boll, 1954a). It was found that the growth factors required for optimal growth for the clone of roots were thiamin, pyridoxin and niacin (Boll, 1954b). It is generally believed that in this system growth is not limited by the synthesis of endogenous metabolites. It may be, however, that these endogenously produced metabolites are inhibitory to growth and that natural antimetabolites are produced endogenously which prevent the toxic action of an excess of the metabolite.

The morphology of an excised root in complete medium is dependent upon: (1) the frequency of lateral root production; (2) the growth rates of main apex and lateral roots relative to each other, and (3) the degree of persistence of the individual meristems. Any factor which, when supplied to the root, tends to alter the morphology of the root is possibly involved with the step by step processes which are responsible for the final morphology of the root.

There is evidence for hormonal, correlative factors, produced within the root, which limit the growth of the main axis or lateral meristems. It has been shown that in excised tomato roots grown in constantly renewed culture medium, the main axis meristem functions for a limited time and ceases activity while the more recently initiated lateral roots are growing rapidly (Street and Roberts, 1952). The duration of activity of this main axis meristem can be prolonged by excision. The hypothesis has been advanced that the inhibition of apical meristematic activity is due

to some influence exerted on the meristem by the main axis and laterals. By excising the apical meristem the source of this inhibitory influence is removed. Street and Roberts have also shown that the main axis meristem and younger parts of the main axis, bearing lateral root initials, exert an inhibitory effect on the growth rate of the young lateral roots. They have shown that by removing the root apex a greater total growth of laterals resulted. Boll (unpublished), has confirmed these results. All of these facts tend to suggest that a hormonal mechanism is involved in the production of the tomato root morphology. The next paragraph will review the evidence which supports the view that an auxin-like substance is actually present in these tomato roots.

An antiauxin α-(1-naphthylmethyl-sulphide)-propionic acid will stimulate the growth of the excised tomato roots (Street, 1954). This evidence tends to suggest that the retardation of growth and cessation of meristematic activity occurring, when excised tomato roots are repeatedly subcultured in standard medium, is due to the accumulation at the apex of some natural hormone to a critical supra-optimal concentration. This hypothesis is further supported by the fact that IAA (3-indolylacetic acid), at concentrations which do not inhibit growth, reduces the survival of these same main axis meristems (Street, 1954). Street (1955) also showed that NAA (1-naphthaleneacetic acid) inhibited excised root growth and, like IAA it had a deleterious effect upon survival. From the above data it is seen that the rate of death is increased by auxin (IAA) and that the survival time is increased by an antiauxin. Therefore, there is evidence for the presence of an auxin-like compound in these tomato roots as was suspected from the excision experiments previously discussed. Furthermore, although the

frequency of production of lateral roots is not increased by IAA it is increased by IAN (Street et al, 1954) and NAA (Boll, unpublished). Other results have indicated that IAA is not found in these roots so it may be possible that IAN or some other related auxin-like compound is involved, as a correlative factor, with the growth mechanisms in these tomato roots.

Boll (unpublished), in some experiments designed to obtain information regarding the physiological and metabolic relationships of methionine in the excised tomato root, has recorded some inhibitory and non-inhibitory effects of ethionine on this growth system. The data indicate that methionine, homocysteine and ethanolamine are related metabolically in these roots as has already been well established in microorganisms and animals (Table V, #5). The work also shows that ethionine exerted a very marked effect upon the morphology of the root, particularly in regard to the ratio of main apex to lateral growth and the variability of this ratio. The frequency of lateral production was increased by ethionine or appropriate concentration ratios of ethionine plus methionine, and these morphological effects varied markedly with the absence and presence of niacin as can be see in Table VIII and by comparing Figure 2 and Figure 3.

It was stated above that the growth of the apical meristem, the formation of the lateral roots, and the relative growth of main axis and lateral meristems, are controlled by hormonal, probably auxin-like, substances. Furthermore, the presence of niacin in the medium might influence the 'auxin' status of the roots because tryptophane is a common precursor of both IAA and niacin (Bonner, 1950). The growth processes involved in the morphogenesis of the excised root, or any other comparable growth system, may be regarded as a series of steps including those involving

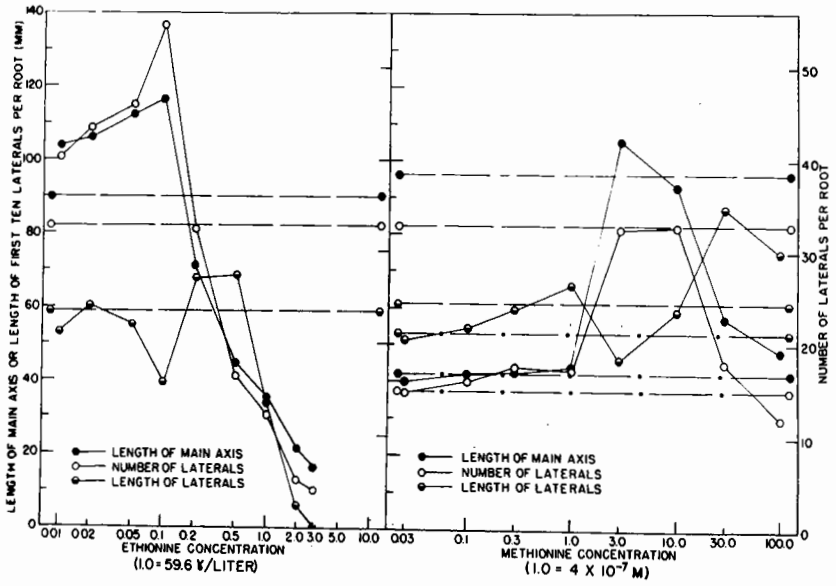

Figure 2 Inhibition of excised tomato roots by ethionine and the reversal of the inhibition by methionine in medium not containing niacin. The horizontal broken lines indicate values obtained with the control medium. Horizontal dot-dash lines indicate values obtained with the control medium plus an inhibitory concentration of ethionine. Each point represents the mean of nine replicates. (Boll, unpublished data)

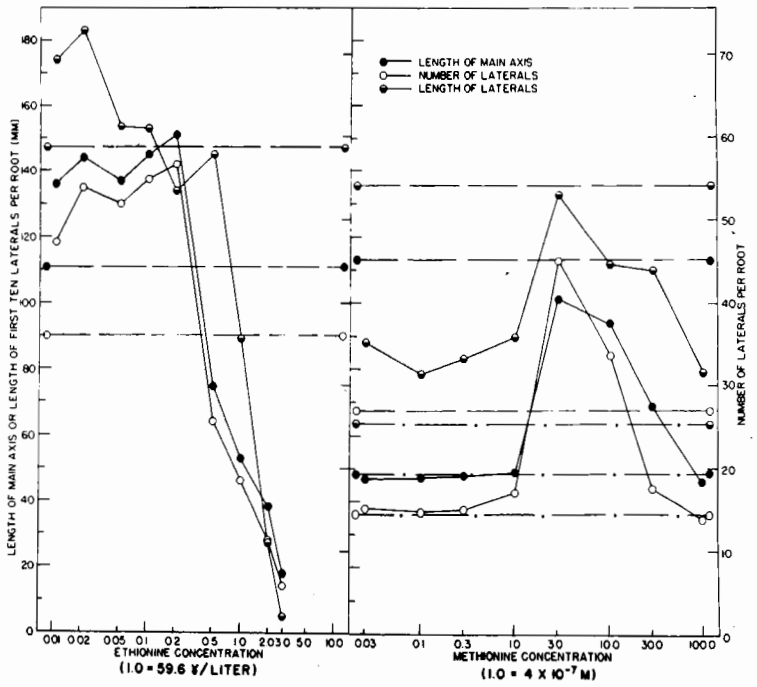

Figure 3 Inhibition of excised tomato roots by ethionine and the reversal of the inhibition by methionine in medium containing niacin. The horizontal broken lines indicate values obtained with the control medium. Horizontal dot-dash lines indicate values obtained with the control medium plus an inhibitory concentration of ethionine. Each point represents the mean of nine replicates.

hormonal mechanisms. Since the same morphological effect might be produced by interfering with any one step, it would be incorrect to consider definitely, that a change in morphology produced by one treatment is indicative of a close relationship between the treatment and the hormonal mechanism. However, in view of the similar effects of ethionine and growth regulators on the excised tomato root, and the dependence of the morphogenetic effect of ethionine on the niacin status, it was felt that the results suggest an unappreciated relationship between the metabolism of methionine and the hormonal mechanism involved in growth of the root. This suspected relationship of methionine (a sulphur containing amino acid) with the hormonal mechanism in the tomato root is of added interest in view of the suggested relationship between 'auxin' and the sulfhydryl groups in pea tissues which was reported recently by Leopold and Guernsey (1953) and Siegel and Galston (1953).

TABLE VIII EFFECT OF ETHIONINE ON LATERAL ROOT FORMATION ON EXCISED TOMATO ROOTS (Boll, unpublished)

In thiamine plus pyridoxin (See Figure 2.)	
Ethionine (gamma/litre)	Number of lateral roots per unit length of length with laterals
0	0.443 ± 0.028^{x} $(9)^{xx}$
5.98	0.530 ± 0.075 (8)
In thiamine, pyridoxin and niacin (See Figure 3)	
Ethionine (gamma/litre)	Number of lateral roots per unit length of length with laterals
0	0.404 ± 0.022^{x} $(8)^{xx}$
11.8	0.486 ± 0.041 (9)

x = Standard Error.
xx = Number of replicates.

The purpose of the research reported in this thesis was to determine the effect of ethionine on other plant growth systems known, or presumed, to involve 'auxin'. The growth systems chosen for treatment were of three kinds:

1) those involving increase in cell size of excised shoot tissue
   a) increase in length of etiolated pea epicotyl sections
   b) increase in diameter of etiolated bean leaf discs

2) those involving growth of shoot tissue (intact) as a product of cell division and cell enlargement
   a) development of lateral buds on green, bean seedlings
   b) growth of hypocotyls of flax seedlings

3) those involving growth of intact roots
   a) rooting of etiolated, excised bean hypocotyls
   b) growth of roots of flax seedlings
   c) growth of roots of wheat seedlings, and in this case, it was possible to measure increase in cell size alone.

Of these systems, number 3a is of particular interest because it was devised especially for use in this project but may be of general application.

It was expected that this variety of growth systems would permit the detection of any general correlation (or otherwise) between the effect of ethionine and the effect of auxin on plant growth. At the same time it was hoped to detect a growth system, simpler than that of the excised tomato root, in which growth stimulation by ethionine might be suf-

ficiently large to permit studies of any interaction, additive or otherwise between auxin and ethionine.[1]

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[1]When the research on the plant growth systems tested here was near completion Schrank (1956), reported that ethionine inhibited the elongation of Avena coleoptile sections and that this inhibition was reversed by methionine. He further showed that ethionine inhibited the curvature of Avena coleoptiles with intact apexes and that the inhibition was reversed by methionine in short term experiments. It would appear that there is some evidence here for the involvment of methionine in these growth systems which are known to be influenced by auxins or plant hormones.

3.0 GENERAL MATERIALS AND METHODS

The etiolated plants used in experiments described here (Sections 4.2, 4.5 and 4.6) were grown, and maintained after treatment, in a light-tight room. There was no strict temperature control but from November until May the temperature in the room was 24°± 2°C.

The plants were grown in polyethylene dish pans (14" x 14" x 3½" deep) or in stainless steel steam-table trays (9" x 12" x 1½" deep). The bottoms of the pans and trays were perforated. Wooden flats, as recommended by Miller (1951), were found to be unsatisfactory for growing these etiolated plants because bacteria and fungi grew on the wood even though the flats were autoclaved before use.

The seeds were sown in silica sand, (fourteen mesh: Dominion Corporation Ltd., Lachine, Quebec). Before use the sand was washed in tap water and autoclaved in enamel pans for 4 hours at 15 pounds pressure. The sterile sand was then partially air-dried. The amount of moisture left in the sand when the seeds were planted depended upon the type of seed to be grown and its moisture requirement. The soaking-time of the seed also varied with the type of seed. This time is indicated within the specific sections.

The pans, except when the plant material was being manipulated, were covered by inverted light-tight boxes of heavy cardboard weighted down with bricks. The edges of the boxes were banded with black, felt ribbon. Tests with photographic paper proved the boxes were light-tight.

In order to reduce fungal and bacterial contamination the room was sprayed at two week intervals with a 4% solution of commercial lysol in water. The floor, and the table and bench tops, which were used for seed pans and work space, were also washed with the lysol solution.

All manipulations in the dark room were carried out in green light. The light source consisted of a Kodak Utility Safe Lamp (Model C) containing a 60 watt, tungsten bulb and a green colour filter (Corning Glass #4-64, 6" x 6").

When etiolated plant material was treated with white light it was placed beneath a bank of eight, 48 inch flourescent lamps (Sylvania 40 watt Cool White - Standard) mounted 1 inch apart on a wooden base covered by aluminum foil. The light source was 23 inches above the plant material giving a light intensity of approximately 700 foot candles at the plant material. Care was taken to place the plant material within a previously outlined area of uniform light intensity.

All glassware used was Pyrex, unless stated otherwise. Every piece of new glassware, except the pipettes, was autoclaved full of water for 6 hours at 15 pounds pressure. Before each use, glassware was washed in a chromic acid bath for 10 minutes and then rinsed ten times in tap water, twice in single distilled water, twice in glass distilled water, shaken to remove large water drops and allowed to air dry. All forceps, glassrods, cork borers, etc. were washed in detergent, well rinsed in the same manner as the glassware, and air-dried.

The distilled water used in these experiments was of two types. The first was single distilled water from a block tin still and the second was

obtained by redistilling the single distilled water in a pyrex glass still. All stock solutions and culture media were made up with double distilled water. The same batch of distilled water was used for all solutions within one experiment.

Reagent grade chemicals were used in all inorganic and carbohydrate stock solutions. All other chemicals used are accounted for in Section 4.0. Stock solutions were kept in a refrigerator at 4°C. The stopper connections were made air tight by a coating of wax. Where necessary the pH of solutions was adjusted with either N/1000 hydrochloric acid or N/1000 and N/10 sodium hydroxide using a glass electrode.

Statistical analyses were made of the data of some experiments. Comparisons of the differences between means were made by the t-test and, where necessary, standard errors are included in the tables. Both the t-tests and standard errors were calculated according to the methods given in Snedecor (1946).

4.0 EXPERIMENTAL AND SPECIFIC MATERIALS AND METHODS

4.1 LATERAL BUD DEVELOPMENT IN DWARF BEANS

Techniques have been described for studying the effect of growth regulators on the development of lateral buds on stems. In all of these techniques the method used for applying the growth regulating substance to the cut end of the stem has differed (Went and Thimann, 1937). In early experiments the regulator was applied directly as a powder. Later it was applied by dissolving it in agar which was then placed on the cut area of the stem (Thimann and Skoog, 1933, 1934). Muller (1935) introduced a method for applying the regulator dissolved in a lanolin paste. A tube method for applying the regulator, dissolved in water, was used by Bentley and Bickle (1952). This method was chosen to study the effects of ethionine on the lateral bud development of the dwarf green beans used here and will be described fully in the following section.[1]

4.11 METHOD

Seeds of Burpee's, Dwarf, Stringless, Green Pod, (Round Pod) Bean were obtained from Dupuy and Ferguson Ltd., Montreal. Unsoaked beans were planted in potting compost, three per 5 inch pot, and placed in an experimental greenhouse. Fertilizer (Rapid-gro) was added to the watering

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[1] After the work for this thesis was started, a new test using etiolated pea seedling sections, bearing lateral buds, was reported (Libbert, 1954). In this test the sections are floated in a petri dish containing a concentration of growth regulator to be tested.

can at 2 week intervals. When the internode above the first true leaves measured 2-3 cm. (approximately 23 days after sowing) the plants were selected for uniformity and one plant was left in each pot. The other two were cut off about 3 cm. from the compost. The selected plants were arranged in groups so that there were equal numbers of each of three height ranges in each treatment group. Each treatment contained eight plants.

In each experiment there were four control groups. The plants of the first group were left intact. In the second group the tops were removed but no tubes were attached. In the third group the tops were removed and the cut end fitted with tubes filled with double distilled water. The fourth group was treated as the third but the tubes were filled with a solution of 15 mg. per litre 3-indolylacetic acid (IAA). All plants in the groups treated with ethionine were decapitated and tubes attached which were filled with the appropriate ethionine solutions.

Before the plants were treated, any remaining cotyledons were removed. The internode between the first true leaves and the second true leaf was then cut, with a sharp razor blade, 0.5 cm. above the node of the first true leaves. A ring of Cello-grease (Fisher Scientific Company, Montreal) was applied at a point about 0.5 cm. below the cut end of the internode. A glass tube was then placed over the cut end and rotated into the Cello-grease forming a water-tight join. Cello-grease was substituted for the lanolin that Bentley and Bickle (1952) used because it had a lower melting point than the lanolin and was thought likely to make a more stable join. Tubes of 2-3 ml. capacity were made from glass tubing of 7 mm. outside diameter and tapered at one end to accomodate stems of different diameters.

The solutions were introduced slowly down the sides of the tubes to prevent the formation of air bubbles over the cut end of the stem. After 8 to 12 hours the tubes were emptied and refilled by means of syringe pipettes. This was repeated once every 24 hours for 7 days. All solutions were stored in a refrigerator at 4°C. and warmed to air temperature before each refilling. After 7 days the lateral leaves were cut off and their lengths were recorded by photographing them or by measuring them separately.

4.12 EXPERIMENTAL

Four experiments were carried out in which Cello-grease was used as the seal for the tubes. The results are summarized in Table IX. Ethionine did not inhibit lateral bud growth at low concentrations. However, in three experiments some stimulation of growth, over that of the water control, was obtained. Figure 4 shows that in Experiment #4 there was a 46.3% increase in the growth of the lateral buds at 60,000 gamma per litre ethionine when compared with the difference in growth between the top on and water controls.

TABLE IX

Experiment	Ethionine concentrations (gamma/litre)	Result
1	0.18, 0.6, 1.8, 6.0, 12	No inhibition or stimulation
2	3,000, 6,000, 48,000, 60,000, 300,000 and 600,000	Small stimulation of growth at 60,000 gamma/litre
3	ditto	ditto
4	48,000 to 72,000	Stimulated at 60,000 and 72,000 gamma/litre (See Figure 4)

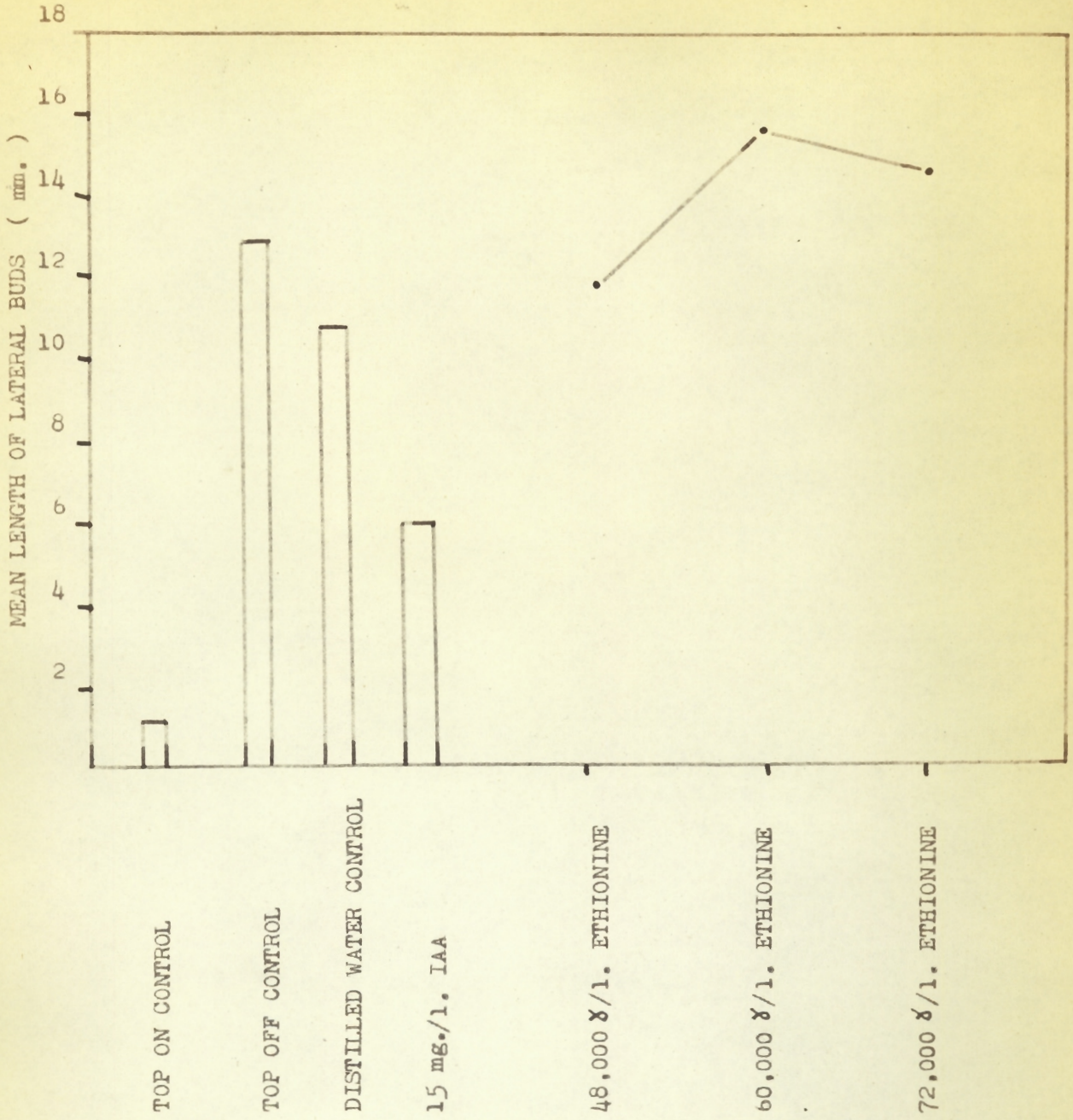

FIGURE 4. THE EFFECT OF ETHIONINE ON LATERAL BUD DEVELOPMENT IN DWARF GREEN BEANS.

Unfortunately in all of these experiments a browning of the stem tissue occurred at the point of application of the Cello-grease. This browning would tend to indicate that there was some oxidizing agent in the grease. If this were so then the stimulation of bud growth by ethionine might be due to a counteraction of an inhibition by the oxidizing agent in the Cello-grease.

In an attempt to find a sealing agent which would remove this oxidation complication, lanolin was purified according to the method given by Redemann, Wittiwer and Sell (1950). The effect of purified lanolin, Cello-grease and non-purified lanolin on growth of bean tissues was then tested by applying them to the cut end of the plants and on the growing petioles. Toxic effects were indicated by the extent of browning and distortion of petiole growth. Contrary to expectation the 'purified' lanolin proved much more toxic than the non-purified lanolin and Cello-grease was between these two.

An experiment was done to test the effect of 48,000 - 72,000 gamma per litre ethionine in lateral bud growth using non-purified lanolin. Unfortunately the low melting point of the lanolin prevented the formation of a tight seal and leakage from the tubes resulted. Despite the indications that ethionine stimulates lateral bud growth the experiments were discontinued due to the difficulties encountered with the sealing grease.

4.2 LATERAL ROOTING OF ETIOLATED BEAN HYPOCOTYLS

Tests have been devised elsewhere which show quantitative relationships between rooting and the concentration of growth regulators. Leopold (1955), mentioned three common methods used for applying growth regulators when testing their effect on the rooting of shoot cuttings; these are the "soak treatment", the "dip treatment" and the "talc treatment". These methods are not easy to control in quantitative studies and are, generally, unsuitable for use in the laboratory.

Went (1934), described a laboratory test using etiolated Pisum seedlings. The method is, briefly, as follows. The shoots, when 10 to 12 cm. long, are cut off just above the first scale-bearing node after which their bases are immersed in a 0.05% solution of potassium permaganate in water for 4 hours. This treatment disinfects the cuttings and improves their keeping quality. According to Curtis (1918), it can also improve rooting. The apical bud is removed and the apical end of the section is split longitudinally. The split end of the section is then immersed in 1 c.c. of the test solution for 15 hours after which the section are removed and placed in a solution of 2% sucrose in water for 7 days. Attempts were made to use this test. It was found to be unsatisfactory because of fungal and bacterial contamination in the sucrose solution. This caused the stems to rot before the roots emerged. From recent correspondence with Dr. Went it has been learned that he has since found the test unsatisfactory.

A rooting test using excised hypocotyls of light-grown dwarf beans was described by Hemberg (1951). The method is, briefly, as follows. Ten day old, sand cultivated plants of Phaseolus Vulgaris L. possessing two full grown leaves and two cotyledons were cut so that the hypocotyl

was 5 cm. long. The cotyledons were removed and the plants were placed one per 100 ml. brown glass jar which was filled with the treatment solution. The jars were placed in boxes covered with glass and containing moist sand. The temperature was maintained at 20° - 22°C and twenty flourescent lamps of 40 watts each served as the light source. The plants were illuminated for 13 hours per day. An attempt to use this test system was made despite the necessity of doing the manipulations in a greenhouse. It also proved unsatisfactory.

The experiments described below were made to determine the suitability of excised, etiolated bean hypocotyls as material for a quantitative rooting test which could be used, in the laboratory, to study the effects of ethionine upon lateral root initiation. It was hoped that in using the etiolated bean hypocotyl for this test there would be, in contrast to the test described by Went, no need to supply external nutrients and, as a consequence, fungal and bacterial contamination in the medium would be eliminated or greatly reduced.

4.21 METHOD

Seeds of Burpee's Dwarf, stringless, Green Pod (Round Pod) Beans (see section 4.11) were treated with a solution of 2% calcium hypochlorite in water for 15 minutes and then rinsed ten times in distilled water. They were then soaked in autoclaved tap water at room temperature for 1.5 hours with one water change every 45 minutes. The seeds were sown about one per square inch on top of a layer of sterile sand, which had been soaked with autoclaved tap water, and allowed to drain for two hours. A 1.5 inch layer of air-dried sand was placed over the bean seeds and the pans were then placed in the dark room (see page 29). All subsequent operations were carried out

in green light (see page 30). After 4 days in the dark the seedlings were watered with sterile tap water.

When most of the hypocotyls were about 10 cm. high (about 5 to 7 days after sowing) the seedlings were removed from the seed pans. After the first two preliminary experiments (see section 4.221) those seedlings with hypocotyls over 12 cm. high were discarded and the remainder were sorted into three groups according to length. These seedlings were trimmed to a length of 5 cm. as measured from the apical side of the bend in the hook. During this trimming operation the seedlings were kept under moist paper towels to prevent dessication.

The rooting tests were made in 1.5 oz. glasses (F.W. Woolworth) 6 cm. tall, 5 cm. deep inside, 5.2 cm. in diameter at the mouth and 4 cm. in diameter at the base. The vessels were of soft glass. When eight hypocotyls were placed in a glass there was a liquid capacity of about 45 ml. Two glasses were used per treatment with eight hypocotyls in each. All treatments contained the same number of hypocotyls from each length group. The hypocotyls were arranged so that the terminal hooks clasped the edges of the glass vessels. In this way, all the hypocotyls were immersed to about the same height in the treatment solutions (Figure 5). The hooks of plants over 12 cm. high were too far open to clasp the edge of the vessel.

4.22 EXPERIMENTAL

4.221 PRELIMINARY EXPERIMENTS TO PERFECT THE TEST

A preliminary experiment was done to determine whether decotylized hypocotyls alone would supply enough nutrients to permit lateral rooting

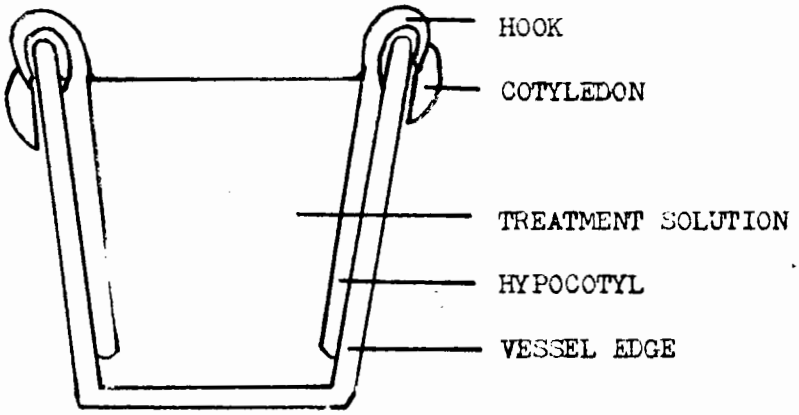

FIGURE 5. A DIAGRAM SHOWING THE ARRANGEMENT OF ETIOLATED BEAN HYPOCOTYLS IN A TREATMENT GLASS. EACH HYPOCOTYL WAS ARRANGED SO THAT THE TERMINAL HOOK CLASPED THE EDGE OF THE VESSEL.

when an appropriate external auxin concentration was supplied, or whether the cotyledons were necessary as a nutrient source. After 4 days in the dark, lateral roots were initiated on the hypocotyls with cotyledons intact and which were in IAA concentrations of 0, 2, 5, 10 and 100 mg. per litre in distilled water. The increase in hypocotyl length was inhibited at the higher IAA concentrations. In these same IAA concentrations the decotylized hypocotyls were stunted and the number of laterals was markedly decreased. Thus in the case of the decotylized hypocotyls the response to IAA was limited by a lack of nutrients.

A second experiment was done to study the effect of intact leaves and/or intact cotyledons on lateral root formation when external IAA was applied in concentrations of 0, 0.1, 0.5, 1.0, 5, 10 and 100 mg. per litre. This experiment showed that the cotyledons alone supply sufficient nutrients to permit a marked response to IAA.

Prior to being placed in the treatment solutions, the hypocotyls were marked according to the height of the seedling from which they were cut. It was found, in all treatments, that hypocotyls cut from seedlings 13 cm. or over did not give a uniform response. Therefore, in all subsequent experiments, those seedlings over 12 cm. high were discarded and the remainder were sorted into three groups according to length.

Some incidental observations were made after leaving the hypocotyls in the light for 5 days following the 4 day dark treatment period. These are of interest and worthy of mention here. (1) The presence of leaves as well as cotyledons permitted hypocotyl length increase in an IAA concentration (100 mg. per litre) which was inhibitory to hypocotyl increase when the cotyledons alone were present. This suggests that the leaves

supply an "anti-auxin" which reduces the activity of the available auxin. (2) Long and branched lateral roots were formed with 0 and 10 mg. per litre IAA only if cotyledons alone were present. The presence of leaves as well as cotyledons inhibited the lateral root length and reduced the amount of branching. (3) The presence of cotyledons promoted chlorophyll formation in the hypocotyl near the attachment of the cotyledons and in leaves when present. If the cotyledons were not present the chlorophyll was not formed. It would appear that the cotyledons supply one or more substances necessary for chlorophyll formation.

In a third experiment, hypocotyls plus cotyledons were treated with IAA concentrations ranging from 0.1 mg. to 50 mg. per litre. The results of this experiment provided an IAA action curve (Figure 6). At 10 mg. per litre IAA, many lateral roots were produced with only slight inhibition of hypocotyl length increase.

The maximum response to exogenous auxin would be expected when endogenous nutrients were not limiting but endogenous auxin was at a minimum. On the assumption that at least part of the endogenous auxin comes from the cotyledons, an experiment was carried out to test the effect of amputating parts of the cotyledons on extension of the hypocotyl and rooting. The stages of amputation are shown in Figure 7. The apical bud was removed in all treatments. At the same time the effect of IAA concentration on the hypocotyls was tested. The results are given in Figure 8 which shows that with progressive removal of the cotyledons there was, in the minus IAA controls, a progressive decrease in the extension growth of the hypocotyls and in the number of roots formed. Furthermore, there was a progressive decrease in the number of roots produced in 10 mg. per litre IAA.

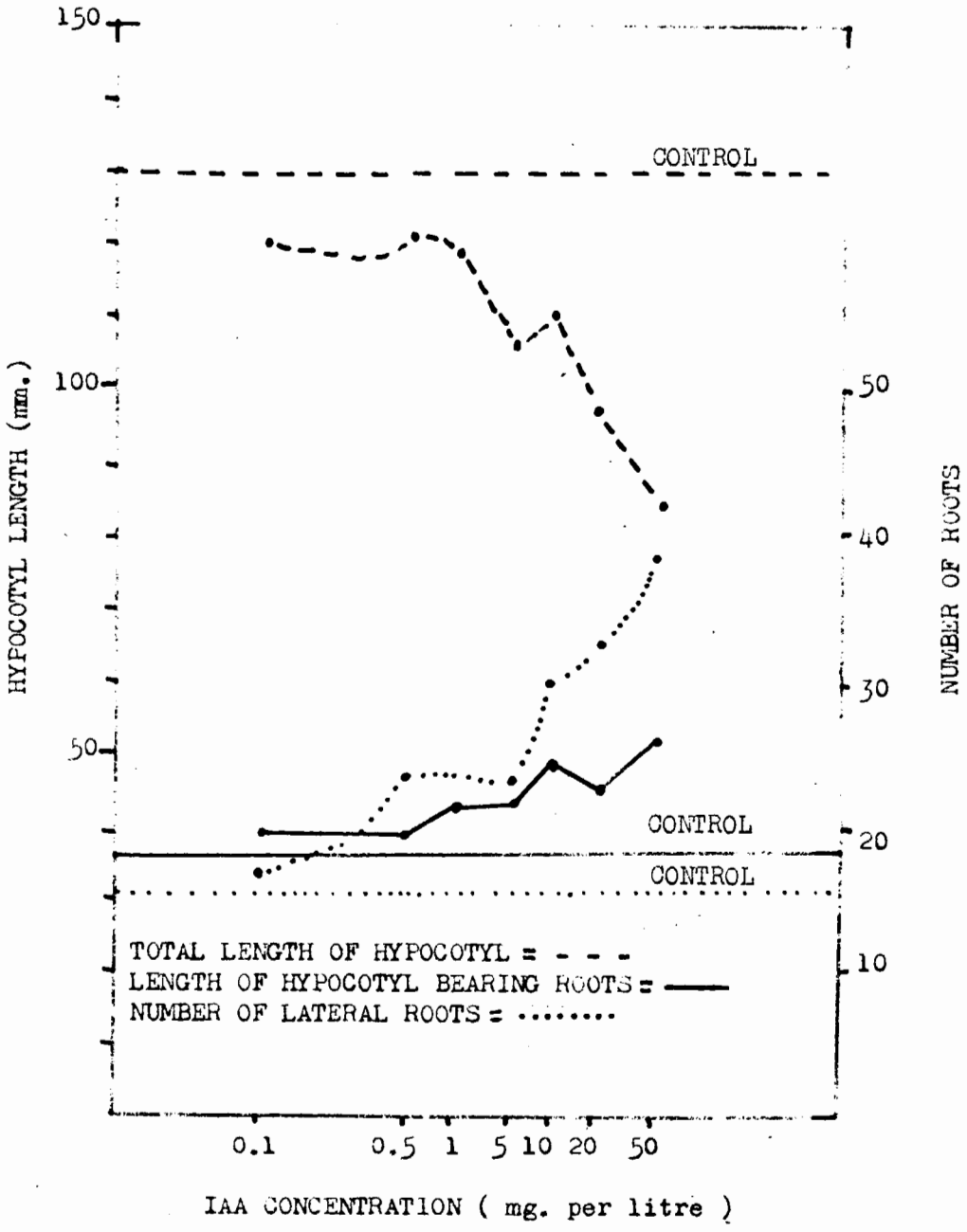

FIGURE 6. THE EFFECT OF AUXIN ON GROWTH AND LATERAL ROOT PRODUCTION OF ETIOLATED BEAN HYPOCOTYLS.

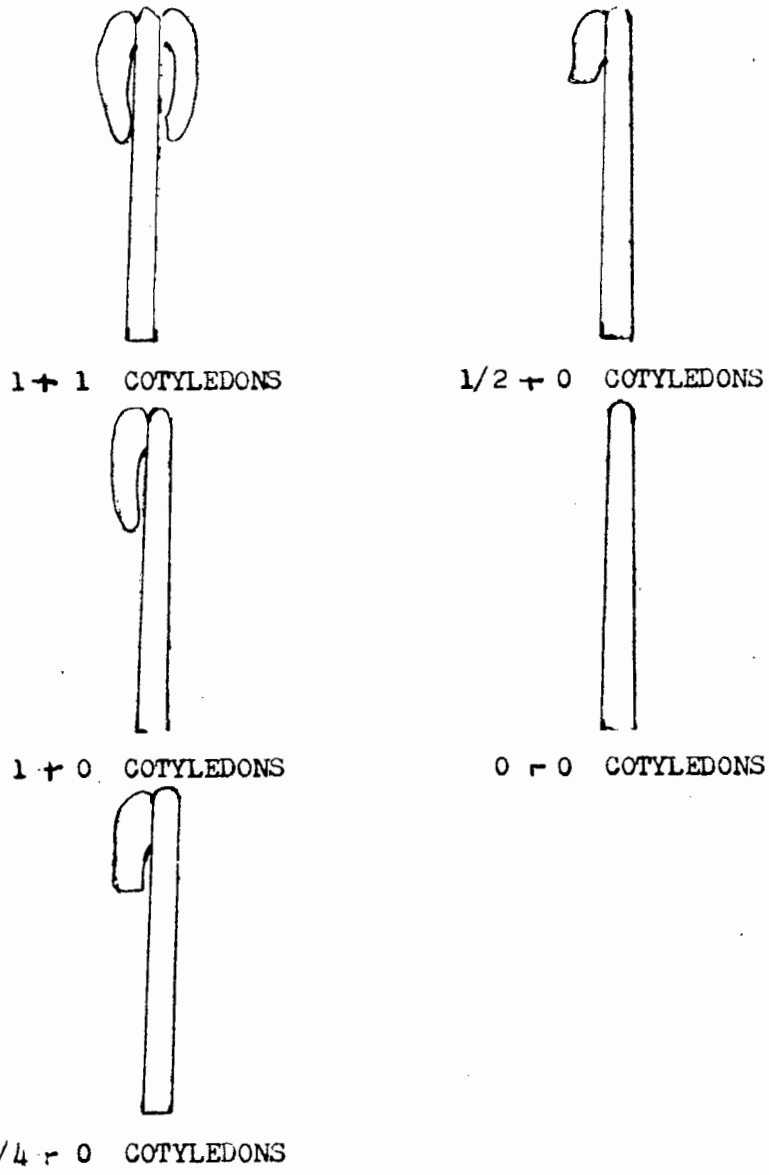

FIGURE 7. A DIAGRAM SHOWING THE STAGES OF COTYLEDON AMPUTATION WHICH WERE TESTED ON EXTENSION AND ROOTING OF THE ETIOLATED BEAN HYPOCOTYL.

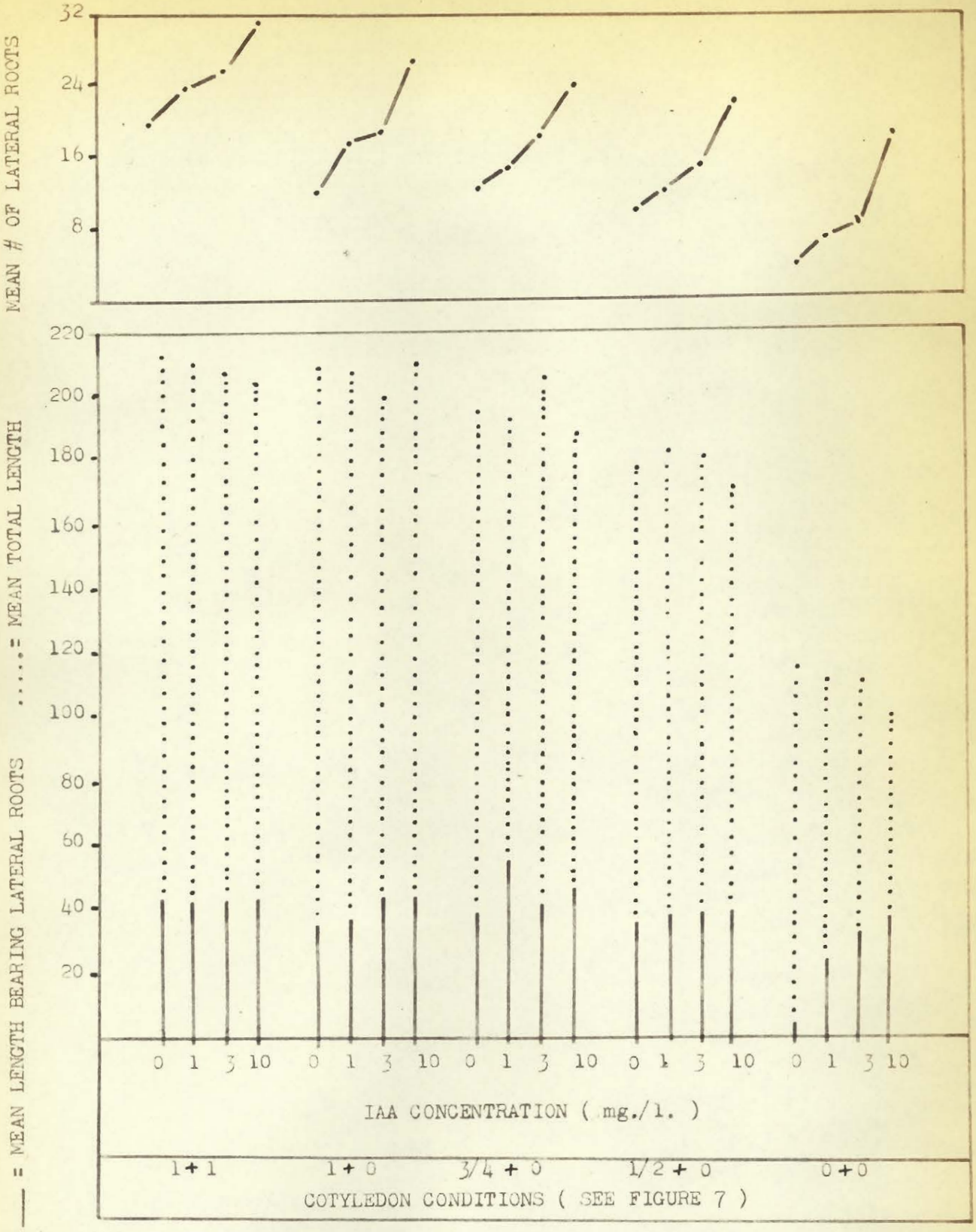

FIGURE 8. THE EFFECT OF AMPUTATING PARTS OF COTYLEDONS ON THE RESPONSE OF THE ETIOLATED BEAN HYPOCOTYL TO IAA.

The amputation of portions of the cotyledons gave little, if any, increase in the rooting response to IAA as measured by the difference between numbers of roots in the control and 10 mg. per litre IAA. However, with less than one whole cotyledon, the extension growth of the hypocotyl was decreased. This indicated that, with less than one cotyledon, endogenous nutrients were limiting growth. The use of a unilateral nutrient source did not appear to affect the distribution of the lateral roots in the hypocotyl.

In view of these facts it was decided that hypocotyls with the terminal bud and one cotyledon removed would be used in the rooting test. The application of this new rooting test to the detection of rooting activity of ethionine is described in the next section.

4.222 EFFECT OF ETHIONINE ON ROOTING

Experiments were designed to test the effect of ethionine dilutions on the lateral rooting response of bean hypocotyls with one cotyledon and also on decotylized hypocotyls. Ethionine dilutions of 1.8 to 18,000 gamma per litre were tested. The effect of 10 mg. per litre IAA was included in each experiment.

In the first experiment the hypocotyls were cut after 5 days and treated. The data indicated that ethionine, at 6 gamma per litre, gave a slight increase in lateral root number over the control in the hypocotyl plus one cotyledon series. The experiment was repeated. The second experiment was done when the seedlings were 7 days old. At this time, the seedlings were the same size as those in the first experiment but a difference in the temperature of the growing room caused the second group to develop more slowly than the first one did. The data showed that there

was an increase of 27% in lateral root number over the control at 60 gamma per litre in the hypocotyl plus one cotyledon series. Furthermore, there was a 13.3% increase in lateral root production at 1.8 gamma per litre ethionine in the decotylized series (Table X).

TABLE X

Ethionine treatment (gamma / litre)	Number of roots per decotylized hypocotyl
0	11.67 ± .749 (18)
1.8	14.65 ± .752 (17)

Probability of the difference $< .01$

4.3 THE GROWTH OF PRIMARY ROOTS OF WHEAT

Root elongation is a complex phenomenon involving two processes, cell multiplication and cell elongation. Both processes must be considered when one attempts to study the effect of substances on root growth.

The growth of roots has been measured in diverse ways. Most workers have simply measured increase in length. In some cases cell measurements, usually of one cell type, were made to help determine the contribution of cell division and cell expansion to the overall growth in length (Torrey 1956). It is incorrect, however, to assume that the length change in one cell type is typical of all the cells. This has been shown by Brown et al (1952) and Wanner (1950). The increase in fresh weight has been used to measure root growth by Audus and Shipton (1952) and Audus and Thresh (1953). Clonal root material in sterile culture has been used to study root growth. Street (1954) and Street et al (1954) have measured root growth in terms of increase in main axis length and in root survival. Boll (1954c, 1955) has measured root growth in this way and also considered the initiation of lateral roots and the total length of the ten basal lateral roots. Because of the ease and speed of measurement, the measurement of root growth in terms of length increase has been used most extensively in testing the physiological activity of growth regulating substances. Whilst recognizing its limitations, the method is used here to study, quantitatively, the effect of ethionine on primary roots. In some experiments the lengths of epidermal cells were also measured.

The first quantitative test involving intact seedling roots was established by Swanson (1946) with Silver King corn. Most of the tests described subsequently are modifications of this technique, generally using other species of plants (Leopold, 1955). These subsequent tests are listed in Leopold (1955) and Torrey (1956). It is interesting to note that Burstrom (1950) has developed a technique in which the main modification to Swanson's method is the use of a constantly flowing test solution. Swanson's technique as modified and used by Lexander (1953) for wheat has been slightly altered and used here.

4.31 METHOD

The seed used in the experiments reported here was Triticum vulgare Vill. var. Thatcher obtained from the Department of Agronomy, Macdonald College, Ste. Anne de Bellevue, Quebec.

The seeds were sown, endosperm down, on filter paper in sterile, 11.0 cm. petri dishes. The filter paper was moistened with 8 ml. of double distilled water before autoclaving. The dishes of seeds were placed in a dark, temperature controlled oven at 26°C. for 48 hours.

After 48 hours, uncontaminated seedlings with straight roots were selected, removed from the germinating dishes, and the primary roots were measured. Seedlings, taken at random, were then transferred to 9 cm. petri dishes containing the treatment solutions. The length of the primary root was marked on the side of the dish beside the seedling to which the measurement belonged.

The basal medium in which the seedlings were grown was that described by Lexander (1953). The composition is as follows:

KH_2PO_4	$3 \times 10^{-4}M$
KNO_3	$2 \times 10^{-4}M$
$Ca(NO_3)_2$	$4 \times 10^{-4}M$
$MgSO_4$	$2 \times 10^{-4}M$
$MnCl_2$	$8 \times 10^{-6}M$
$ZnSO_4$	$6.2 \times 10^{-6}M$
H_3Bo_3	$3.2 \times 10^{-7}M$
H_2MoO_4	$1.24 \times 10^{-6}M$
$Fe_2(SO_4)_3$	$2.5 \times 10^{-6}M$

All media were autoclaved for 10 minutes at 15 pounds pressure and were cooled to room temperature before they were pipetted into the appropriate treatment dishes. Each treatment consisted of three dishes with three seedlings in each dish. Each dish contained 4.0 ml. of medium. The treatment dishes plus the seedlings were placed in an incubator at 26° 0.5°C for 24 hours after which time the primary roots were measured. The roots were then removed from the seedlings and were placed in a fixative (2 distilled water :2 ethyl alchol :1 glycerol) and stored in small vials until the cell lengths were to be measured.

About one hour before the cells were to be measured, the excised primary roots were pumped in the fixative to remove air bubbles. One root was then placed as straight as possible on a slide and covered with a 1:1 mixture of Javex (sodium hypochlorite) and distilled water. One hour later a coverglass was placed carefully over the root. Care was taken to prevent

disarrangement of the epidermal cells by not applying pressure to the cover glass. The measuring had to be done before the root had been in the Javex for two hours because after this period of time the cells were disarranged and it was impossible to distinguish the epidermal cells. The effect of the fixative and cleaning agent on cell length of wheat roots cannot be determined because it is not possible to measure the unfixed and uncleared cells of wheat roots. However, the fixative is known to have little or no effect upon the size of hypodermal cells of the tomato root (Boll, unpublished data).

Groups of ten epidermal cells were measured in areas at 15, 20, and 25 mm. from the root tip by means of a Spencer Research Microscope with a X44 achromatic objective and X10 oculars, an eyepiece micrometer and a graduated mechanical stage.

4.32 EXPERIMENTAL

In a preliminary experiment, the effect of ethionine at concentrations of 0.1, 1.0, 5, 10, 50, 100 and 500 gamma per litre was tested on the growth of the primary roots of wheat seedlings. Growth in length was increased by ethionine at 10 gamma per litre. This experiment was repeated three times and the results of all four experiments are given in Figure 9. In one of the repeat experiments a stimulation of root growth, over that of the control, occured at 5 gamma per litre ethionine. In the other two experiments the growth of ethionine treated roots did not surpass that of the controls. A combined graph representing the mean growth as per cent of the mean growth of the four controls is shown in Figure 10. This graph shows that there was a 10% stimulation of primary root growth at 10 gamma per litre ethionine.

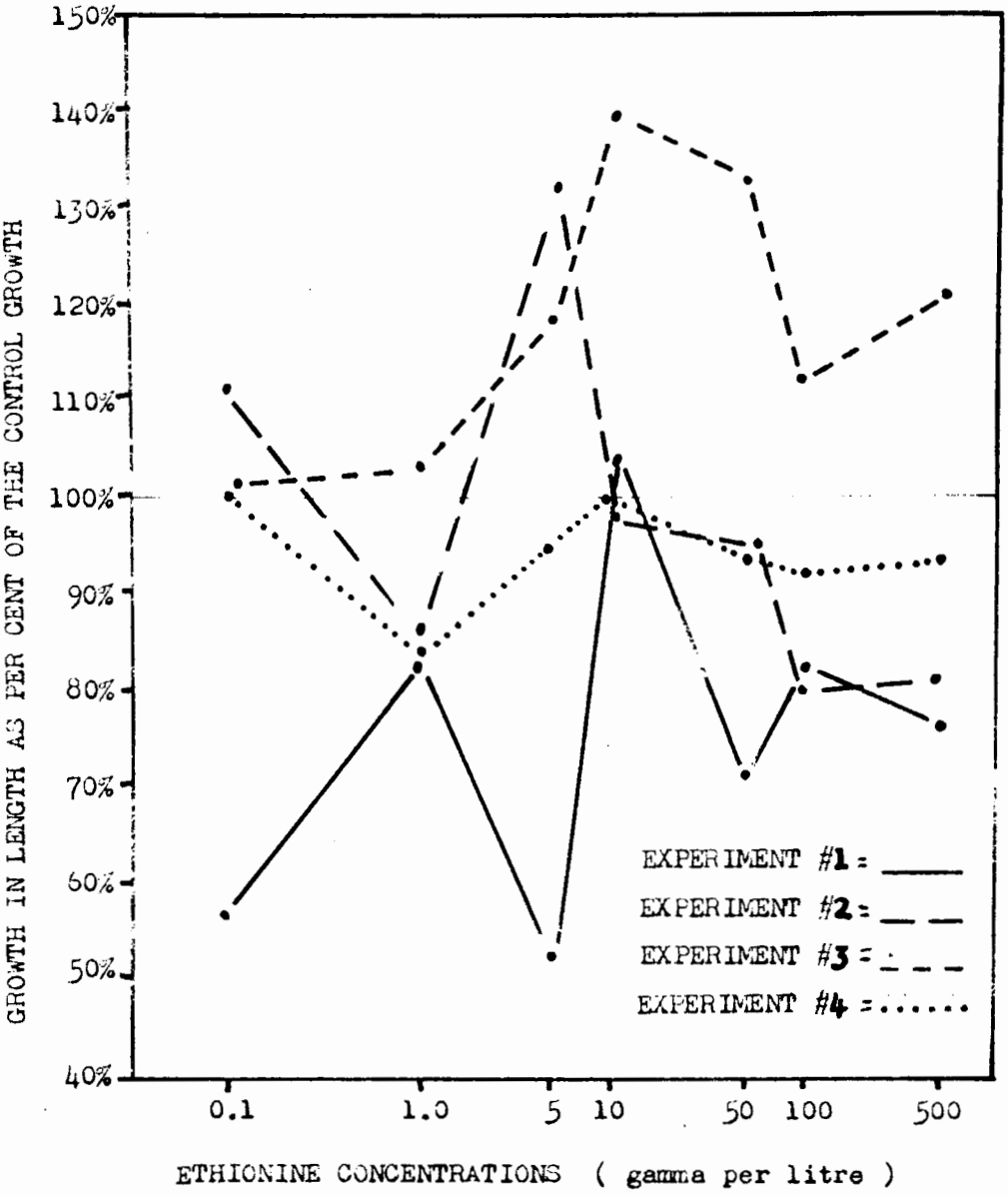

FIGURE 9. THE RESULTS OF FOUR EXPERIMENTS SHOWING THE EFFECT OF ETHIONINE ON THE GROWTH OF PRIMARY ROOTS OF WHEAT.

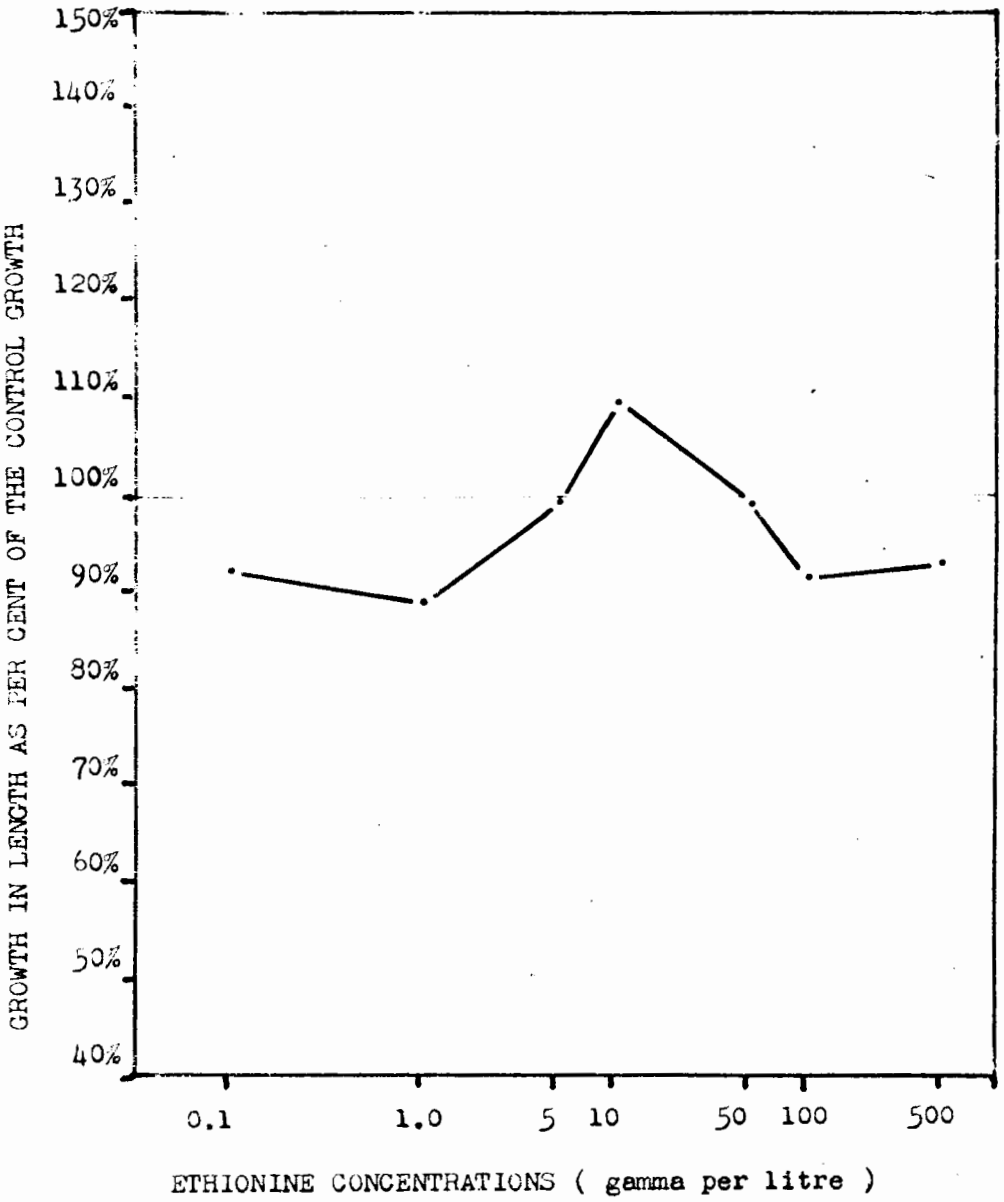

FIGURE 10. A COMBINED GRAPH OF THE RESULTS SHOWN IN FIGURE 9. FOR ANY ONE CONCENTRATION OF ETHIONINE THE RESULTS OF THE FOUR EXPERIMENTS ARE COMBINED AND THE MEAN IS EXPRESSED AS PER CENT OF THE MEAN GROWTH OF THE CONTROLS.

To determine whether the stimulation of root growth in the 10 gamma per litre ethionine treatment was due, to any extent, to a stimulation of cell elongation the experiment was repeated a fifth time and the epidermal cell lengths were measured in selected areas of the root (see Section 4.31). The results are given in Figure 11 which shows the cell length was increased considerably by 10 gamma per litre ethionine. It would appear that the over-all increase in root length, above that of the control at 10 gamma per litre ethionine was due, at least in part, to a stimulation of cell elongation.

The failure of ethionine to stimulate growth in length of the roots in all experiments is puzzling. Although all efforts were made to keep the conditions constant from one experiment to another it was obvious that when the seedlings were selected for treatment they were not at the same stage of development on each occasion. It is possible that response to ethionine varies with the 'physiological age' of the seedling root and that in some instances any stimulation of increase in cell length by ethionine was offset by an inhibitory effect on cell division. For the purposes of this thesis, it is sufficient to record that ethionine, under some conditions, increases the final size of epidermal cells in the roots of wheat seedlings.

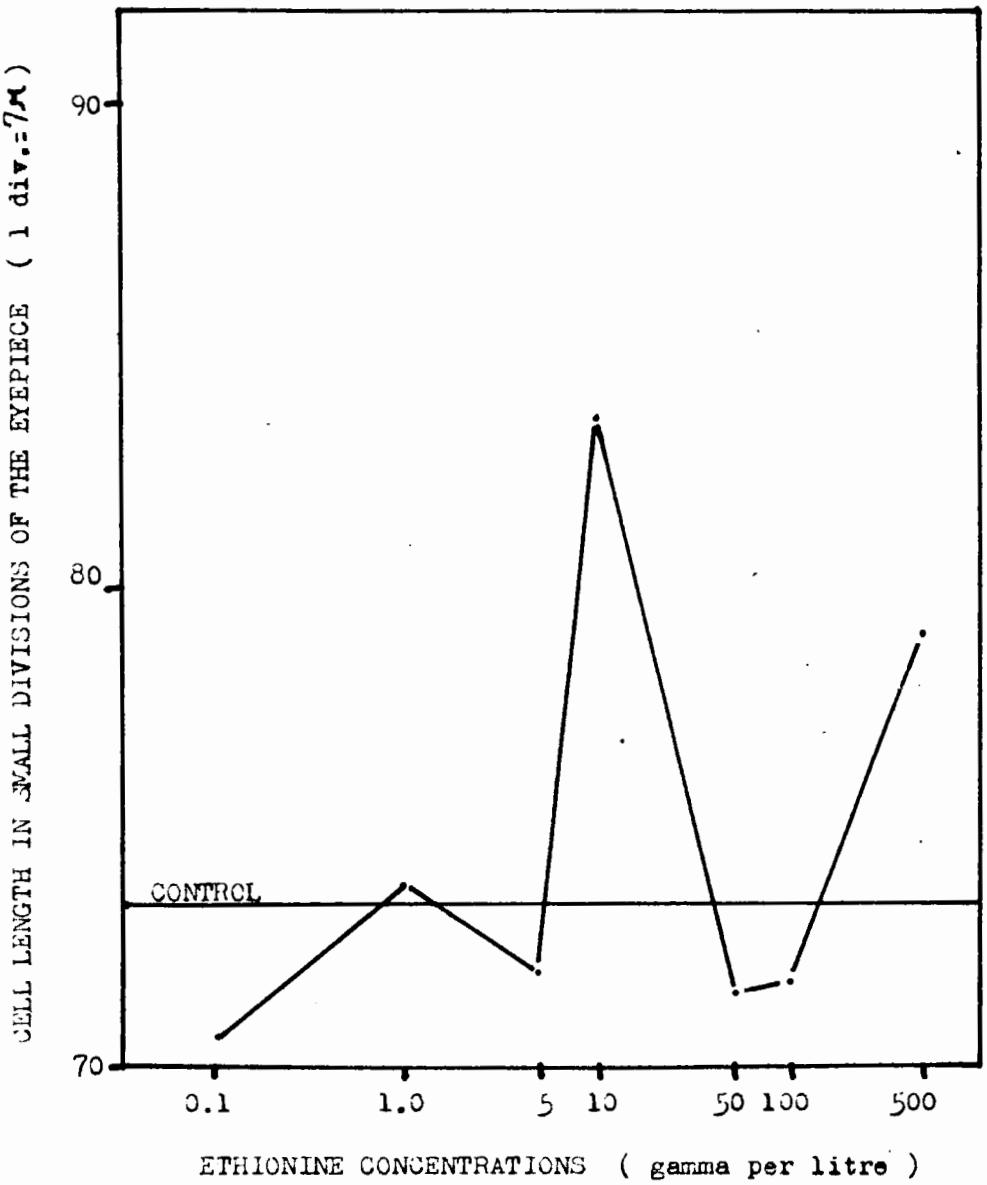

FIGURE 11. THE EFFECT OF ETHIONINE ON THE FINAL LENGTH OF EPIDERMAL CELLS OF THE PRIMARY ROOTS OF WHEAT.

4.4 THE GROWTH OF HYPOCOTYLS AND ROOTS OF FLAX SEEDLINGS

Quantitative tests designed to study the effect of growth regulating substances on seedling growth, particularly the growth of roots, have been listed elsewhere (Section 4.3). These were modifications of the method of Swanson (1946). A particularly effective modification of this method was that of Audus (1951) which involved placing cress seedlings on the rim of a cylinder of filter paper in a glass tube containing the solution to be tested. This modification was of value because the roots grew straight, they were easily measured, and repeated measurements could be made at intervals through the glass without disturbing the test plants. The method used here to test the effect of ethionine dilutions on the growth of the hypocotyl and root of flax (Linum usitatissimum L.) seedlings is a modification of the method of Audus.

4.41 METHODS

The seed used in the experiments reported here was obtained from Dale Laboratories Incorporated, Montreal. They were germinated in the dark room in a cylinder 17 cm. in diameter and 25 cm. long which was lined with filter paper moistened with autoclaved tap water. The cylinder was closed at its open end by a glass plate and was sealed tightly with lanolin paste.

After 48 hours the seedlings were selected for uniformity and distributed at random, eight to a treatment chamber. The treatment chambers comprised a covered dish 8 cm. in diameter and 4 cm. deep. Within this a piece of plate glass 7 cm. wide and 8 cm. high, which was covered with filter paper, rested on the bottom at a 30° angle. The filter paper dipped

into the 10 ml. of solution. Eight seedlings were arranged on the filter paper in a row approximately 4 cm. from the base of the glass plate. The basal medium in which the seedlings were grown contained 1% sucrose and 17 mg. per litre calcium sulfate ($CaSO_4$). During the treatment time, the dishes were kept in a closed glass chamber lined with wet paper towels.

The seedlings were measured with a millimeter ruler at one, two or three days, or on all three days when growth curves were being determined.

4.42 EXPERIMENTAL

The effect of ethionine concentrations of 1, 5, 10, 50, 100, 200, 500, 800, 1000, 5000, 10,000, 15,000 and 20,000 gamma per litre were tested on the growth of the hypocotyl and the root of the flax seedlings. In eleven out of sixteen experiments the hypocotyl showed greater or lesser stimulation. In six out of thirteen experiments the root showed greater or less stimulation. In one experiment 50% inhibition of radical growth occurred at approximately 500 gamma per litre ethionine.

Some experiments were done to compare the effects of ethionine on seedlings growth in the light as opposed to the dark but the results obtained were inconclusive.[1]

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[1] Since the termination of the experiments for this thesis, a test to study the effects of ethionine on the growth of isolated flax root tips was done by Boll (unpublished). In this test 10 mm. root tips were excised from two day old flax seedlings and were treated in 9 cm. petri dishes which contained the same basal media as for the seedling tests above. The root tips were placed above the treatment solution on a piece of glass which was covered with filter paper. Eight tips were treated in each dish for 24 - 48 hours. The control growth was 5 mm. in 24 hours and 6 mm. in 48 hours. The ethionine treatments included concentrations of 1, 5, 10, 50, 100, 500, 1000, 5000, 10,000, 15,000 and 20,000 gamma per litre. An apparent stimulation of the growth in root length occurred at approximately 50 gamma per litre ethionine.

4.5 ETIOLATED PEA SECTION STRAIGHT GROWTH TEST

According to Leopold (1955), "the physiological basis for straight growth tests is the simple stimulation of straight growth by auxins". In such tests there are no limitations to the transport of the substance to be tested and there is no necessity for elaborate equipment. These two facts make the straight growth tests popular for testing the effect of growth inhibitors on cell size increase.

A straight growth test using pea stem sections was first described by Thimann and Schneider (1939). Other workers have used this test with slight modifications to study the effects of various compounds on cell size increase. Included among these are Galston and Hand (1949), Miller (1954), Nitsch (1955), and Schrank (1956). The straight growth test using etiolated pea sections has been slightly modified for use under the McGill laboratory conditions.

4.51 METHOD

The pea seeds used in the experiments reported here were Pisum sativum L. var. Alaska purchased from Dupuy and Ferguson Ltd., Montreal.

The seeds were soaked for 4 hours in autoclaved tap water in the dark. They were sown by the same method used for the Dwarf Green beans (see section 4.21), except that they were covered with one half an inch of dry sand instead of the partially air-dried sand.

When the third internode was 1 - 2 cm. long (after approximately 6 days) the seedlings were harvested and the sections were cut just below the hook with a section cutter giving an initial length of approximately

7.4 mm. The sections were stored in a 9 cm. petri dish containing distilled water until the cutting operation was over. They were thoroughly mixed prior to distributing them among the treatment dishes.

The sections were treated in 6 cm. petri dishes containing 5 ml. of solution and 10 sections per dish. The basal medium was 2% sucrose and the pH was adjusted to 5.5.

The sections were measured against a millimeter plastic ruler on the stage of a dissecting microscope. They were measured to the nearest 0.5 mm. by using, in addition to the ruler, a graduated ocular in the microscope.

4.52 EXPERIMENTAL

The effect of ethionine, at concentrations of 0.1 - 25,000 gamma per litre, was tested on the length increase of etiolated pea hypocotyl sections. Concentrations of 0.1 and 1.0 mg. per litre IAA were included in each experiment and the experiment was repeated four times. The results are given in Figure 12 which shows that, from one experiment to another there was a considerable variation in response to both IAA and to ethionine. However, in experiment #5 the stimulation of growth by ethionine at 10 gamma per litre was significant with a probability of 0.05. By comparing the results of these experiments it can be seen that, generally speaking, when the response to auxin was high, then ethionine stimulated the growth but when the response to auxin was low then ethionine inhibited growth.

In an attempt to eliminate the variability in response to IAA some experiments were done to test the effect of various pre-treatments on the response to IAA. The pre-treatments included pre-soaking the sections in

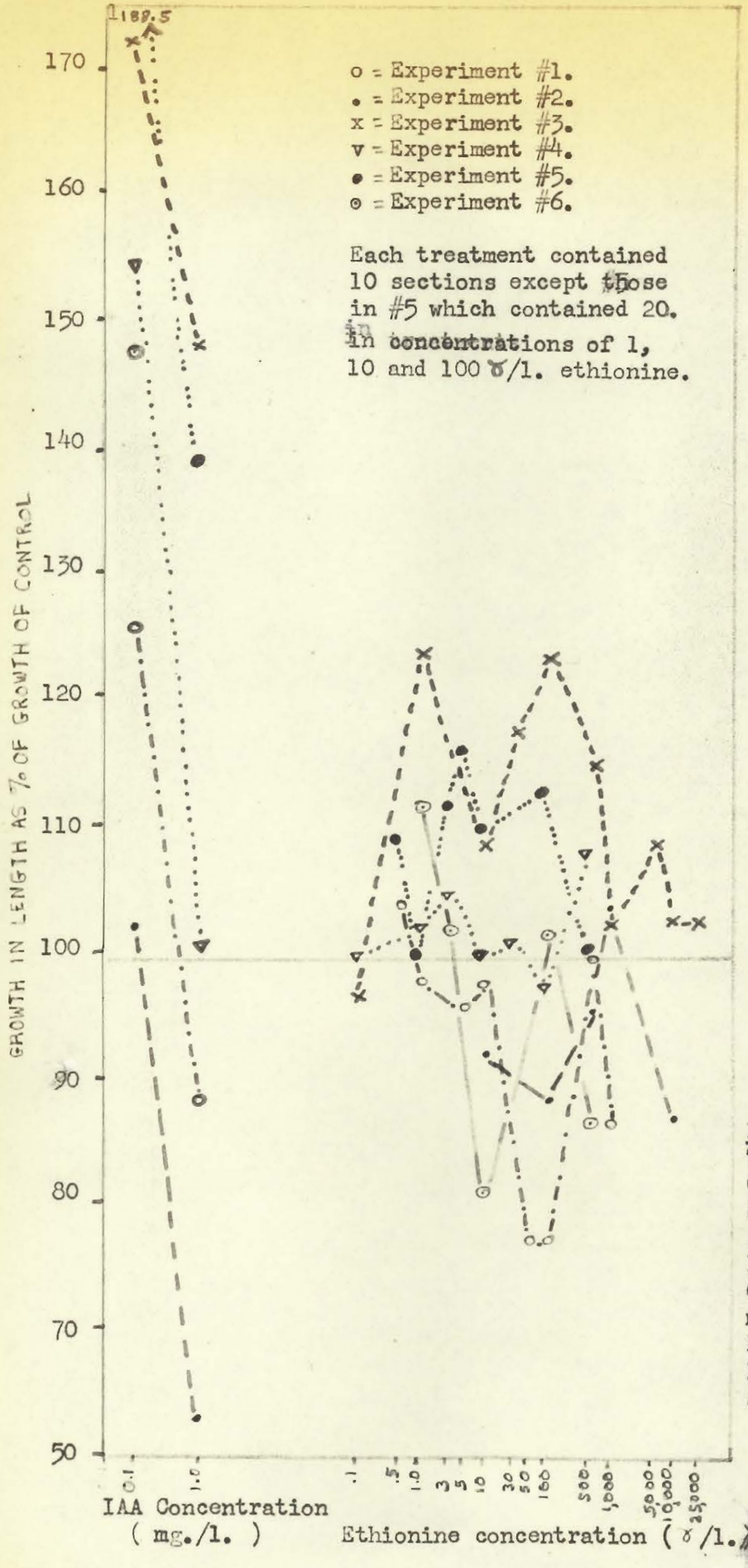


FIGURE 12.
THE EFFECT OF ETHIONINE ON THE GROWTH OF ETIOLATED PEA HYPOCOTYL SECTIONS (FIVE EXPERIMENTS ARE INCLUDED). THE EFFECT OF IAA AT 0.1 AND 1.0 mg./l. IS INCLUDED FOR EACH EXPERIMENT AND IS FOUND ON THE LEFT HAND SIDE OF THE GRAPH.

distilled water for 1, 2 or 3 hours or in 1 mg. per litre manganese sulphate ($MnSO_4.H_2O$) for 3 hours or exposing the seedlings or sections to red light (Kodak, Wratten, Series One, Filter) for durations of 15, 30 or 90 minutes. All of these treatments are believed to reduce the effect of endogenous auxin (Nitsch and Nitsch, 1956). None of the treatments gave any consistent increase in the reliability of the response to IAA.

In one experiment the effect of ethionine on sections pre-soaked in manganese sulfate solution ($MnSO_4.H_2O$ 1 mg. per litre) was tested. The results showed that manganese lowered the response to a suboptimal concentration of IAA and also lowered the response to the ethionine concentrations (Figure 13).

As has been mentioned previously, the response of the sections to ethionine appeared to be correlated with the response of the sections to IAA. In view of this an experiment was done to test the effect of the ethionine concentrations in the presence of three auxin concentrations. The results of this experiment are shown in Figure 14. It will be noted that, in the three auxin concentrations, the response to ethionine treatment was very variable. One point is noteworthy. At 500 gamma per litre ethionine in 0.1 mg. per litre IAA there was a definite inhibition of the section growth. In this same ethionine concentration but in the presence of 0.01 IAA mg. per litre there was no inhibition of the section growth. It would appear that there was an interaction between ethionine and IAA at 500 gamma per litre ethionine.

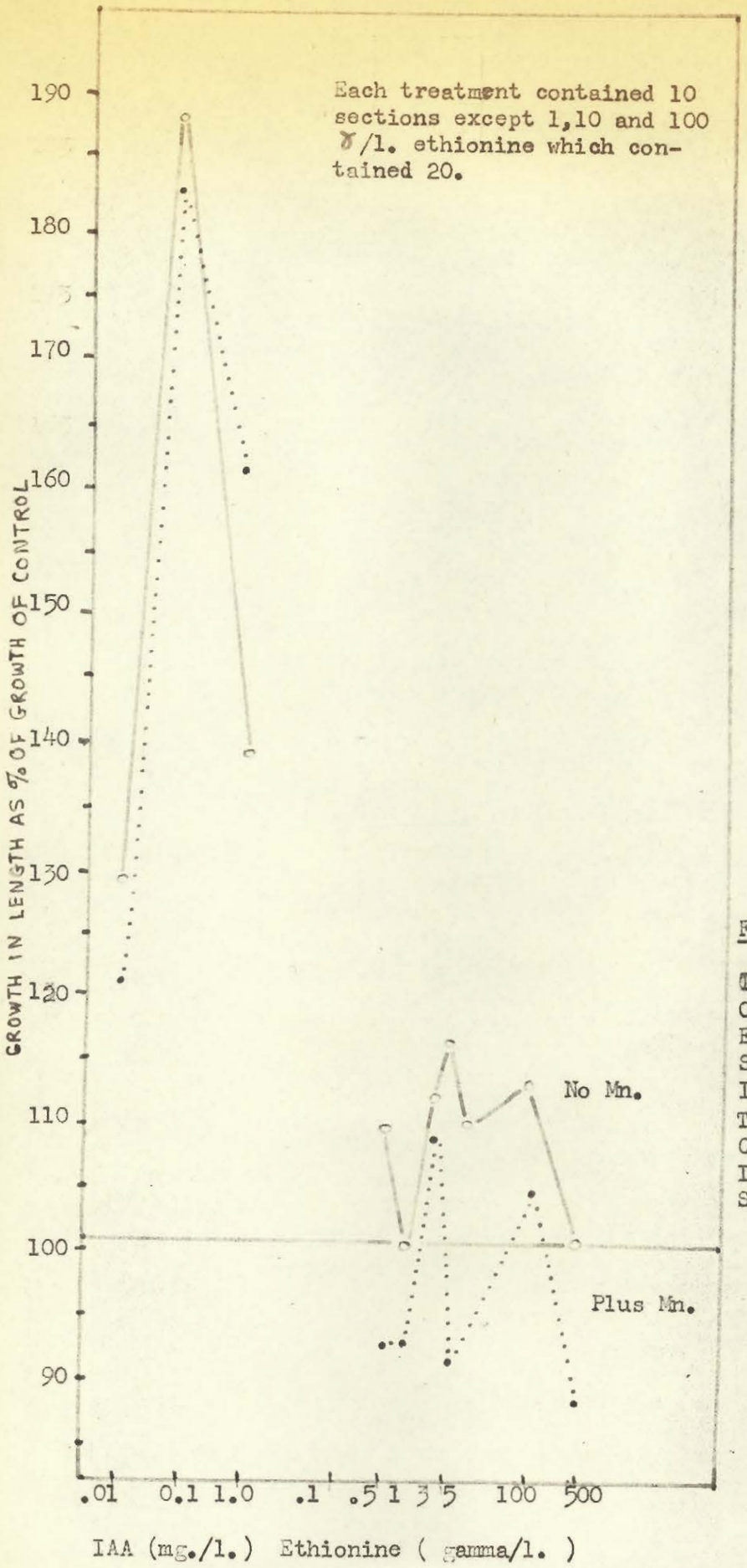


FIGURE 13.

THE EFFECT OF ETHIONINE ON GROWTH IN LENGTH OF ETIOLATED HYPOCOTYL SECTIONS AFTER PRESOAKING IN $MnSO_4.H_2O$ (1mg./l.) THE EFFECT OF IAA AT THREE CONCENTRATIONS IS ALSO INCLUDED AT THE LEFT HAND SIDE OF THE GRAPH.

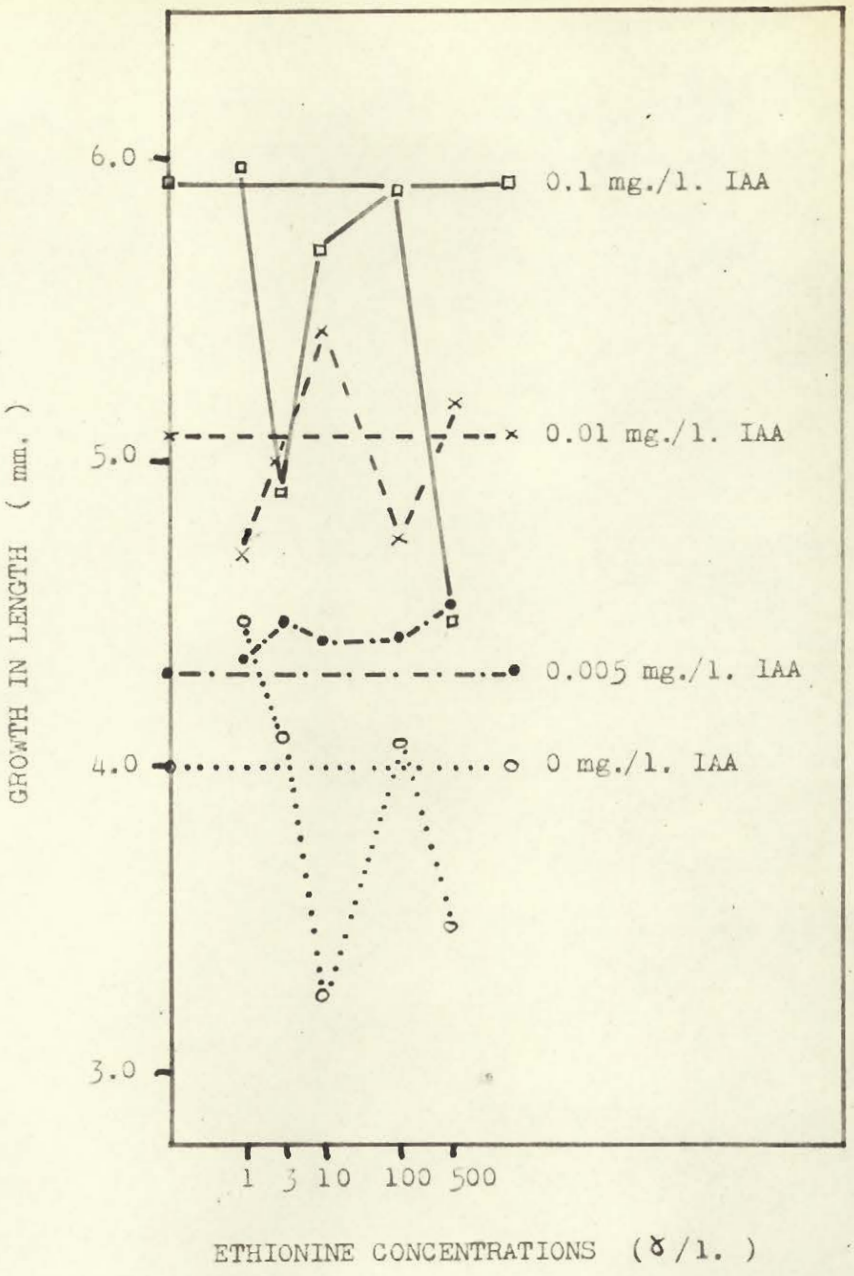


FIGURE 14. THE EFFECT OF ETHIONINE, AT THREE AUXIN CONCENTRATIONS, ON GROWTH IN LENGTH OF ETIOLATED PEA HYPOCOTYL SECTIONS.

4.6 THE EXPANSION OF ETIOLATED BEAN LEAF DISCS

If etiolated plants, in which leaf size is suppressed, are exposed to light for a brief period of time a greatly enhanced expansion of the leaves occurs. It is apparent that a photoreaction other than photosynthesis occurs in the leaves and this photoreaction influences the expansion of etiolated leaves. Miller (1951) has devised a test, using discs punched from etiolated leaves, to study this photoreaction and other related reactions involved in the increase in the cell size of leaf tissue.

The etiolated leaf disc test is a relatively simple one for which little elaborate equipment is needed. Discs are cut from the etiolated leaves and placed on the bottom of a petri dish lined with filter paper and containing a basal medium composed of D-glucose and potassium nitrate. The manipulations are done in green light. After three days in the dark the discs are removed and their diameters are measured. The expansion is nearly uniform in all directions.

Miller (1951) found that the exposure of the discs to white light stimulated the growth of the tissue beyond the limited amount which occurs in the basal medium in the dark. He also found that treatment in the dark with cobalt salts would stimulate the expansion of the discs. In a subsequent paper (Miller, 1952) he showed that this tissue increase was due to cell size increase which was nearly uniform throughout the disc. Miller suggested that the light and the cobalt actions were influencing the same processes because in all the plant materials which were tested including: (a) etiolated Burpee Dwarf Stringless Greenpod beans, (b) young green pea

plants (var. Little Marvel), (c) green leaves of Chenopodium album and (d) green leaves of radish (var. French Breakfast) one effect could not be observed unless the other effect occurred as well. It was also shown that light and cobalt were additive in their effect on the expansion of etiolated bean leaf discs. This fact could be a point in favour of the argument that light and cobalt are affecting the same part of the physiological mechanism of cell expansion.

It would appear from Miller's work that the leaf disc test, which he devised, is valuable for observing the effect of growth regulating substances on cell size increase. These observations can be made with or without the influence of the light effect. By such observations it may be possible to determine what steps are involved in the processes of cell size increase and which of these are dependent upon a light effect. Miller's leaf disc test was used here to study the effect of ethionine on cell size increase.

4.61 METHOD

Burpee beans (Section 4.11) were sown and grown as described for the lateral root initiation test (Section 4.21). When the plants were about 31.0 cm. high (about 11 to 14 days after sowing, depending upon the temperature) the leaves were removed and placed, until all the discs were cut, in a deep petri dish lined with moist filter paper. The discs were cut with a brass cork borer 0.5 cm. in diameter. Two discs were cut from each leaf, one from each side of the mid-vein at the base of the leaf. Each disc included a lateral vein approximately at the diameter of the disc. A glass rod was used to remove the discs from the cork borer. About sixty to

eighty discs were placed temporarily in petri dishes lined with moist filter paper until all the cutting was completed.

After all discs had been cut, they were distributed into 9 cm. treatment petri dishes. Each treatment dish received the same number of discs from each storage dish. The discs were placed, upper epidermis down, on top of two pieces of filter paper previously moistened with 5 ml. of the treatment solutions. Two dishes with eight discs in each were used for each treatment condition unless stated otherwise.

The basal medium contained D-glucose (3%) and potassium nitrate (0.08 M). In any one experiment a maximum of four controls were included. These were:

1) Basal medium plus green light
2) Basal medium plus white light treatment
3) Basal medium plus cobalt sulfate (5 p.p.m.) plus green light
4) Basal medium plus cobalt sulfate (5 p.p.m.) plus white light treatment.

The ethionine concentrations ranged from 0.18 to 18,000 gamma per litre. The media were not autoclaved and pH was adjusted to 5.6.

After the discs were placed in the treatment dishes they were exposed to white light for a time which varied from one experiment to another. For the description of the white light source see page 30. The discs were grown for 4 days in the dark and then measured with an ocular micrometer in a binocular microscope. Before measuring, the discs were blotted on filter paper and then placed between two microscope slides held together with elastic bands. The ends of both slides were bound in Scotch Tape to prevent the discs from being crushed between the slides.

4.62 EXPERIMENTAL

The experiments reported in the following section were designed to test whether the stimulation of expansion of leaf discs by light, cobalt and light plus cobalt, as reported by Miller (1952), could be reproduced here. In subsequent sections the effects of ethionine and various growth-active substances are reported.

4.621 THE EFFECT OF LIGHT, COBALT AND LIGHT PLUS COBALT

The effect of increasing exposure to light, in the presence and absence of cobalt sulfate at 5 p.p.m., is shown in Figure 15. The results are similar to those reported by Miller. Discs without light treatment expanded slightly and a light exposure of as little as one minute gave a marked increase in the expansion. With longer light exposure the expansion increased slightly and continued to increase up to the longest light treatment used (30 minutes). The curve was linear between one minute and 30 minutes. The graph also shows that expansion in the absence of light was increased by cobalt and that the increase due to light and cobalt together was more than additive.

The effect of cobalt concentration on expansion of discs given a light treatment of 60 minutes is shown in Figure 16. The curve is similar to that obtained by Miller with the optimum at 5 p.p.m. Cobalt sulfate was inhibitory at higher concentrations.

In subsequent experiments reported here the cobalt sulfate concentration was 5 p.p.m. and the light exposure was 5 minutes unless stated otherwise.

The average final diameter of 5 mm. discs obtained by Miller are shown in Table XI. These can be compared with the various experiments

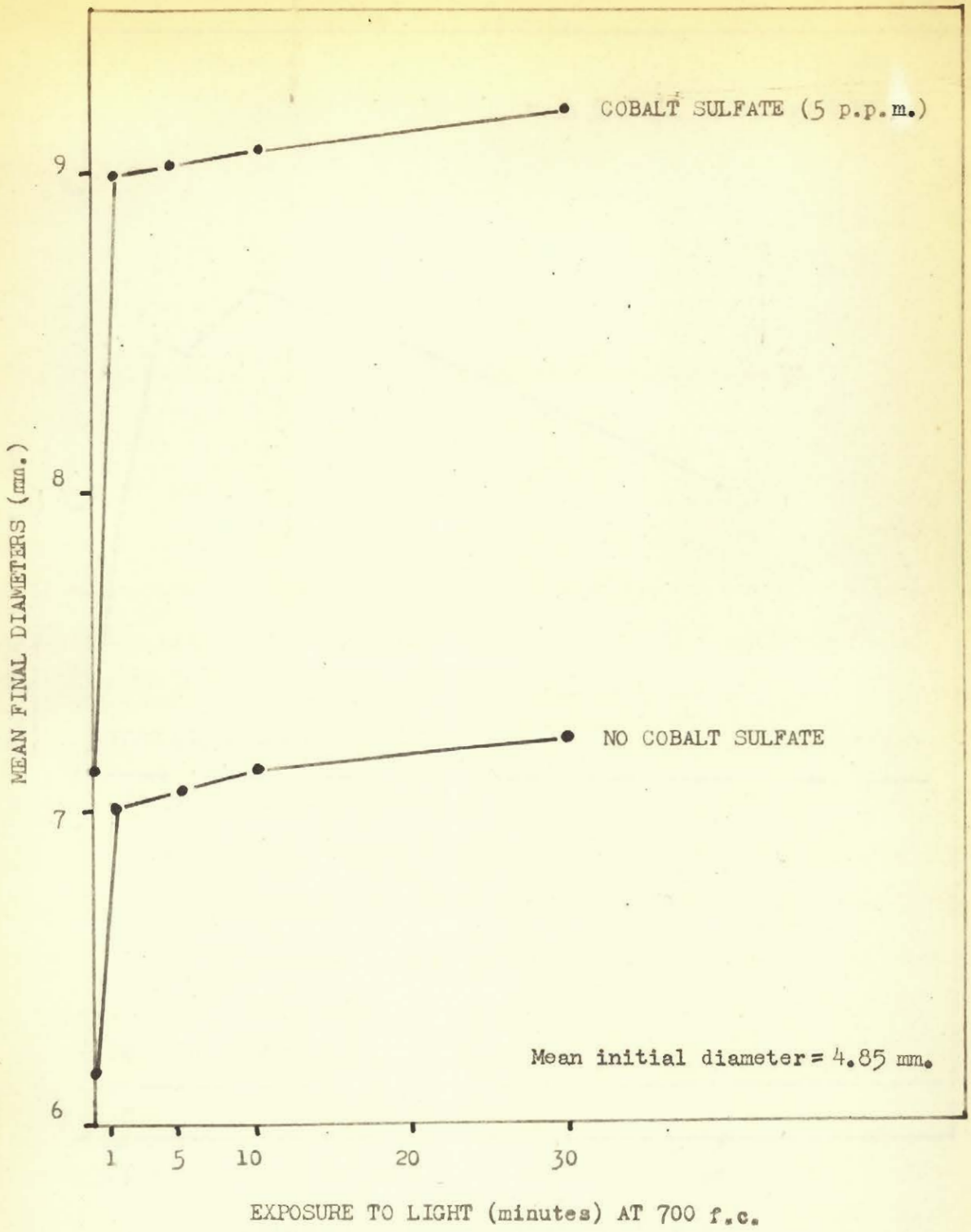


FIGURE 15. THE EFFECT OF EXPOSURE TO LIGHT, IN THE PRESENCE AND ABSENCE OF COBALT SULFATE, ON THE EXPANSION OF ETIOLATED BEAN LEAF DISCS.

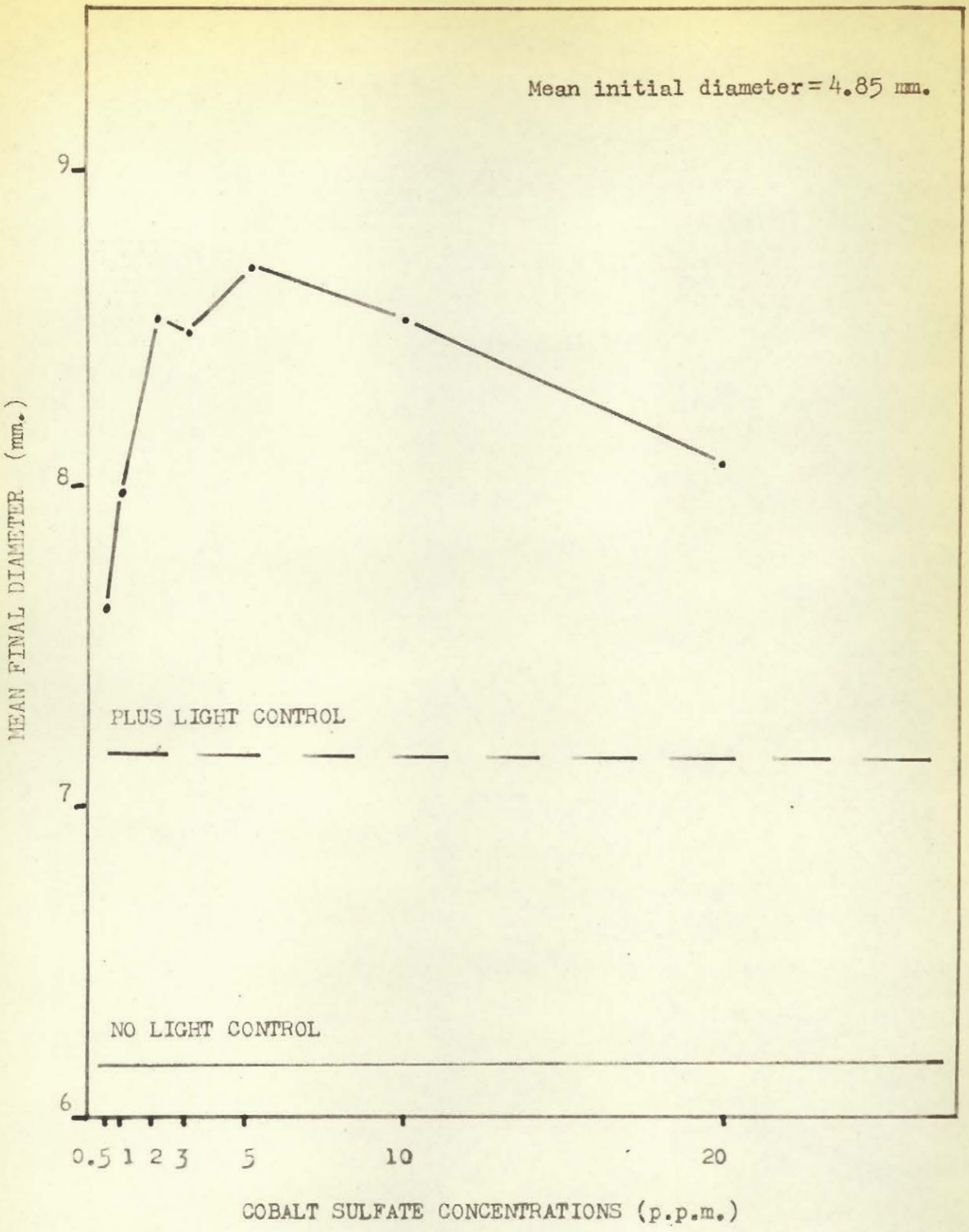


FIGURE 16. THE EFFECT OF COBALT SULFATE ON EXPANSION OF ETIOLATED BEAN LEAF DISCS GIVEN A LIGHT TREATMENT OF SIXTY MINUTES.

reported here. In general, the growth of the dark controls was on the order of that obtained by Miller. Growth with light, cobalt, or light

Table XI

Disc treatments	Mean final diameter (mm.) (Miller 1952)
Dark control	6.6
Light control	8.1
Cobalt & dark	8.3
Cobalt & light	9.7

plus cobalt was generally somewhat lower than that obtained by Miller. This was possibly due to a difference in the conditions under which the plants were grown in the two labs. The responses obtained were considered sufficient to justify the use of the test to study the effects of ethionine on cell size increase in leaf tissue.

An incidental observation was made after the discs had been measured and left in the light for 24 hours. The discs which had been treated with cobalt did not develop chlorophyll as the other discs had done. Cobalt appeared to inhibit the process of chlorophyll production. An experiment, illustrating this effect with a range of concentrations of cobalt, is shown in the Kodachrome transparency (Figure 17).

4.622 THE EFFECT OF ETHIONINE

Three experiments were done to test the effect of lower ethionine concentrations of 0.18, 0.6, 1.8, 6, 18 and 60 gamma per litre on leaf disc expansion in the absence and in the presence of a light treatment. No significant effect was obtained either in the light or the dark in any

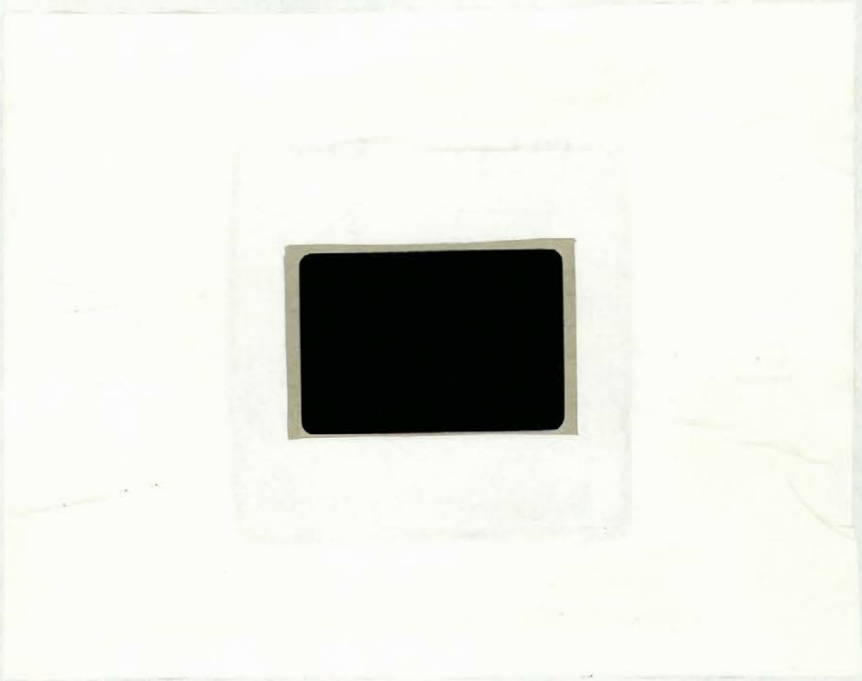


FIGURE 17. KODACHROME TRANSPARENCY SHOWING THE INHIBITORY EFFECT OF COBALT CONCENTRATION UPON CHLOROPHYLL PRODUCTION IN BEAN LEAF DISCS.

A = DARK CONTROL

B = LIGHT CONTROL

C_1 = 0.5 p.p.m. COBALT SULFATE

C_2 = 1.0 p.p.m. COBALT SULFATE

C_3 = 2.0 p.p.m. COBALT SULFATE

C_4 = 3.0 p.p.m. COBALT SULFATE

C_5 = 5.0 p.p.m. COBALT SULFATE

C_6 = 10.0 p.p.m. COBALT SULFATE

of these experiments although in one of them a slight inhibition of the light stimulation was produced by 1.8 gamma per litre.

Two preliminary experiments were carried out to test higher concentrations of ethionine in the presence and absence of light. The concentration range was 60 to 18,000 gamma per litre. In both of these experiments ethionine at 600 gamma per litre increased the expansion of discs treated with light. There was no effect in the absence of light. The results of one of these experiments are given in Figure 18. In order to confirm the stimulation of light-treated discs by ethionine, a third experiment was carried out in which a larger number of replicates per treatment was used and the results were analyzed statistically. The results are given in Table XII. Standard errors for the light control and light plus 800 gamma ethionine are included and numbers of replicates are given in brackets. The difference between the means of the light control and light

Table XII

800 gamma/litre ethionine plus light (eyepiece divisions)	Light control (eyepiece divisions)	P
3.868±0.0505 (36)	3.675±0.0355 (32)	< .01

1 eyepiece division=1.88 mm.

plus 800 ethionine was compared by a t-test and found to be highly significant ($P < .01$). Thus it is concluded that ethionine stimulated the cell expansion in these etiolated leaf discs.

Some incidental observations were made after the discs of the second of the first two preliminary experiments had been measured and left in the light for 24 hours. Ethionine concentrations of 180 gamma per litre and above were found to inhibit chlorophyll formation. It is interesting that

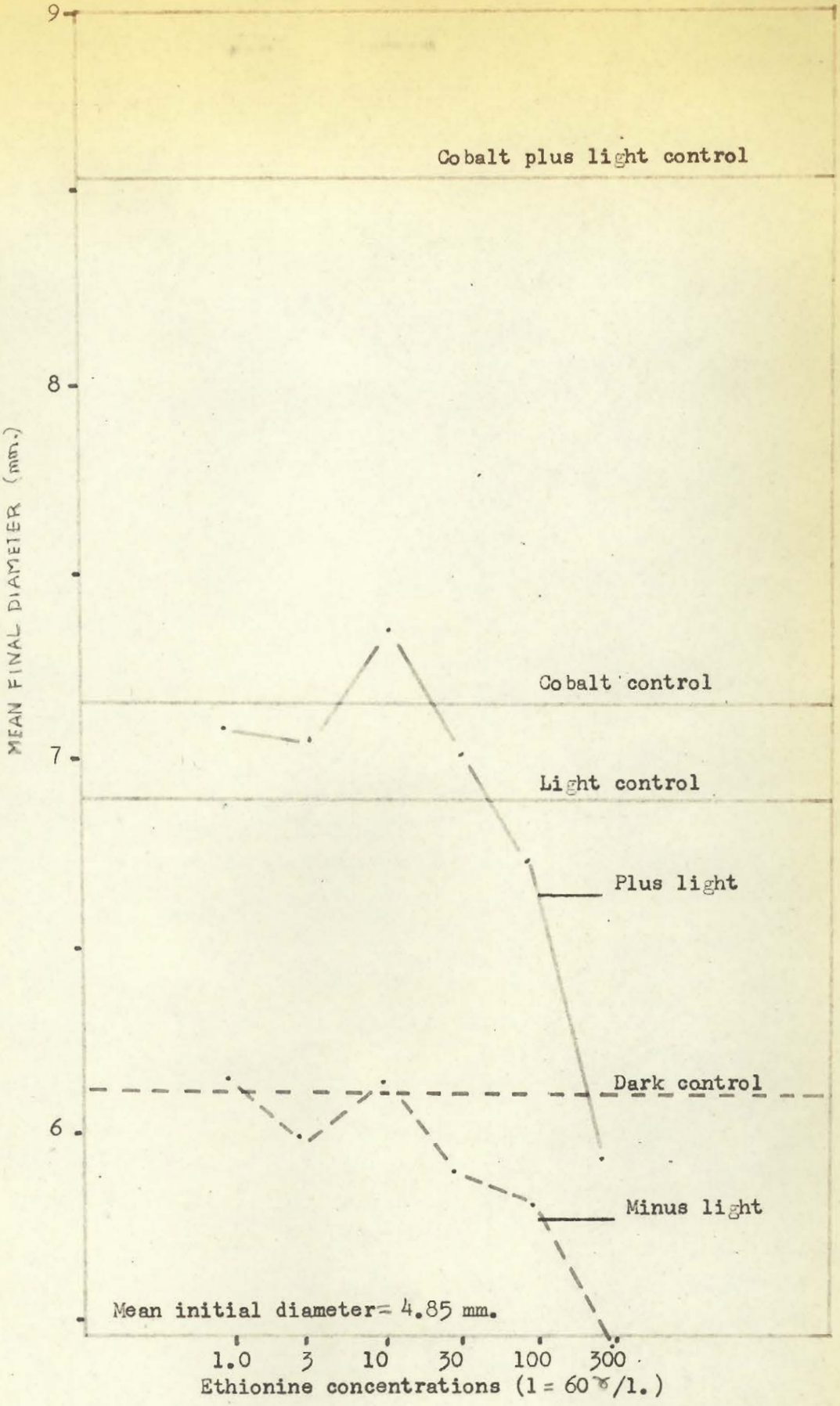


FIGURE 18. THE EFFECT OF ETHIONINE ON ETIOLATED BEAN LEAF DISC EXPANSION WITH AND WITHOUT LIGHT TREATMENT.

both ethionine and cobalt (Section 4.621) have been shown to inhibit chlorophyll formation in view of the fact that they both stimulate disc expansion after a light treatment.

In view of the stimulatory effect of ethionine, following light treatment, it was decided to examine whether ethionine and light, or ethionine and cobalt, showed additive effects comparable to the additive effect between light and cobalt in the expansion of the leaf discs (Miller, 1952).

Figure 19 shows the effect of a range of concentrations of ethionine on leaf discs treated with light for either 30 minutes or 90 minutes. The increase in growth with the longer light treatment in the absence of ethionine, as shown in Figure 16, is confirmed here. Ethionine stimulated expansion with both light treatments. The interpretation of the results is complicated by the fact that 180 gamma per litre ethionine stimulated expansion with the short light treatment but not with the long light treatment. Furthermore, ethionine at 900 gamma per litre was inhibitory with the short light treatment but this inhibition was counteracted by the long light treatment. However, on the basis of a comparison of the effect of ethionine in the two light treatments, there was an additive stimulation between light and ethionine at 300 gamma per litre ethionine. The results indicate that there was interaction, though complicated, between ethionine and the light controlled growth process of the leaf discs.

Figure 20 shows the effect of a range of concentrations of ethionine treated with light (4 minutes) and 0, 1.3 and 5.0 p.p.m. cobalt sulfate. Ethionine stimulated expansion in the absence of cobalt. However, ethionine, if anything, decreased the stimulatory effect of cobalt following light

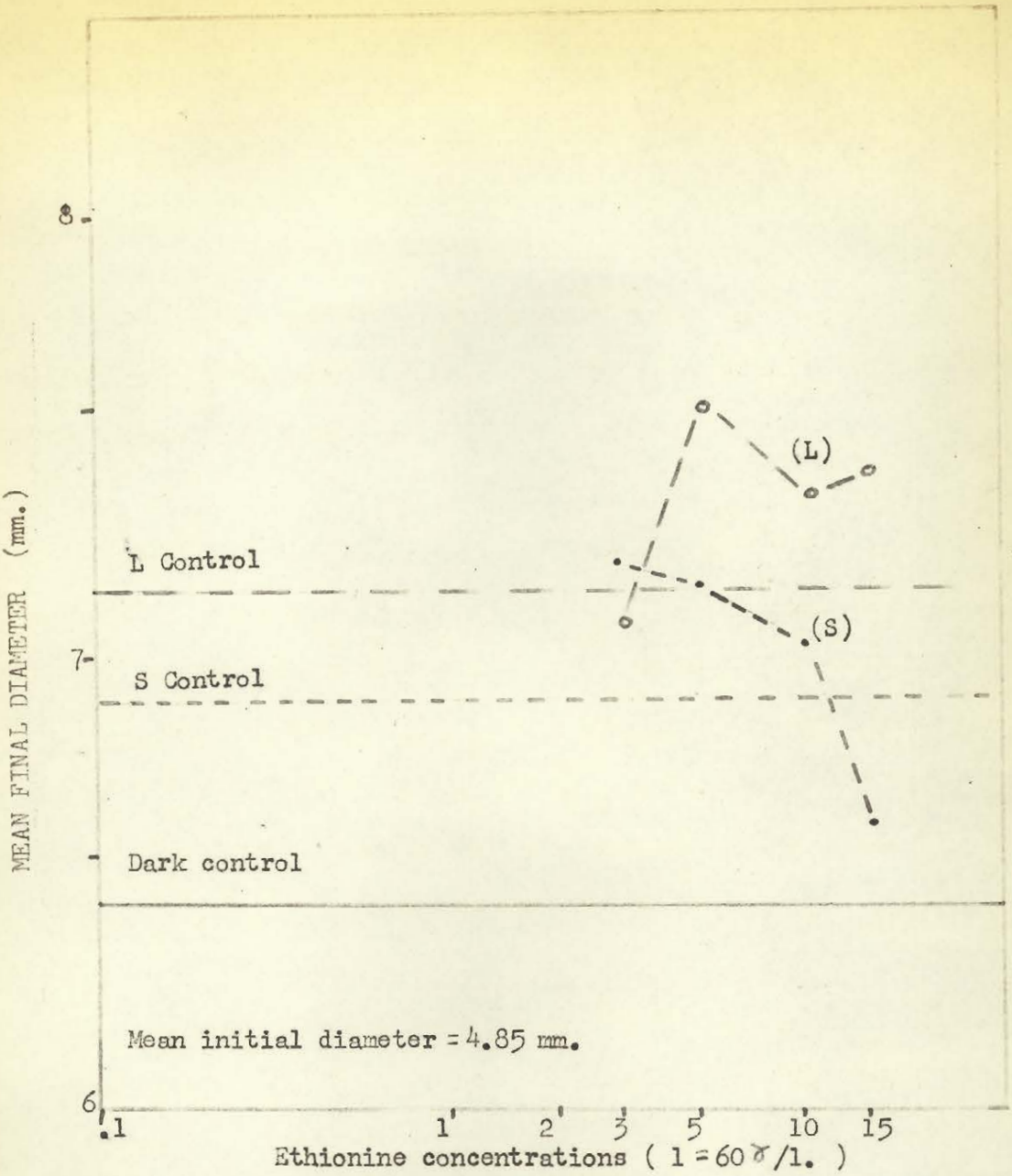


FIGURE 19. THE EFFECT OF ETHIONINE ON EXPANSION OF ETIOLATED BEAN LEAF DISCS TREATED WITH LIGHT FOR EITHER THIRTY (S) OR NINETY (L) MINUTES.

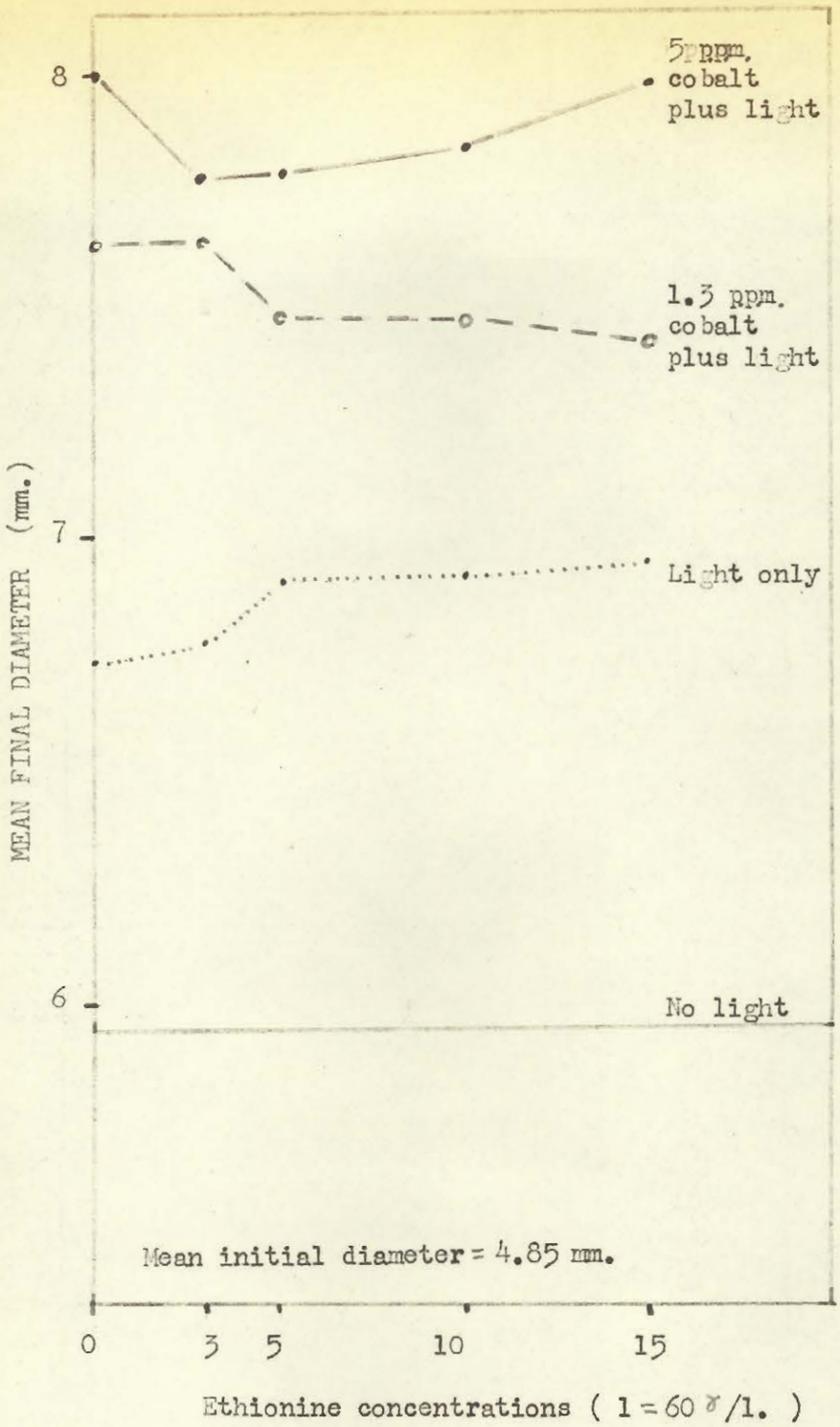


FIGURE 20. THE EFFECT OF ETHIONINE ON ETIOLATED BEAN LEAF DISCS TREATED WITH LIGHT (FOUR MINUTES) AT TWO CONCENTRATIONS OF COBALT SULFATE.

treatment. This is in contrast to the type of interaction between light and ethionine shown in the previous experiment. Amino acids are known to act as chelating agents. Thus the effect of ethionine on the stimulation by cobalt might be explained if ethionine acts as a chelating agent making the cobalt unavailable to the leaf disc.

4.623 EFFECT OF SOME GROWTH REGULATOR, PENICILLIN G AND ETHYLENEDIAMINETETRAACETIC ACID

It was suggested in the introduction that the morphogenetic effect of ethionine on the excised tomato root might be a consequence of an interference with sulfhydryl (SH) metabolism. Theories of auxin action (Leopold and Guernsey, 1953; Siegel and Galston, 1954; and Muir and Hansch, 1955) include the idea that auxins combine with SH groups of some protein system mediating growth. Similarly the effect of certain antiauxins, for example, maleimides (van Overbeek et al, 1955) and triodobenzoic acid (TIBA), according to Leopold and Price (1956), is attributed to the combination of the antiauxin with such -SH groups. Liverman (1955) states that IAA does not consistently stimulate the expansion of the bean leaf disc and it is possible that if IAA (auxin) is involved in the expansion of the discs it is present in supra optimal concentrations. It was felt that a study of the effect of some antiauxins and the substance Penicillin G, which is believed (Cavallito, 1946 and 1947) to interfere with sulfhydryl metabolism, might throw some light on the question of whether ethionine stimulates growth of the discs as a consequence of an effect on sulhydryl metabolites which can be affected by growth regulators. These experiments are reported below together with an experiment in which the effect of ethylenediaminetetraacetic acid (EDTA) was studied.

The effect of EDTA (Bersworth Chemical Co., Framingham, Mass.) was tested, at .01, .03, 0.1, 0.3, 1.0, 3, 10, 30 and 100 mg. per litre on the expansion of leaf discs given a light treatment (5 minutes) and in the absence of cobalt. A slight increase in expansion of the discs occurred at 3 mg. per litre EDTA but the results, at noninhibitory concentrations were too erratic to attribute any significance to this slight increase. The EDTA was inhibitory at 10 mg. per litre and higher concentrations. It was concluded that the results do not support the view that the stimulatory effect of ethionine could be attributed to chelating properties.

Penicillin G (U.S.P. Nutritional Biochemicals Corporation) was tested in two experiments on the expansion of light-treated (5 minutes) leaf discs in the absence of cobalt. In the first experiment the concentration range was .01, .03, .1, 13, 1, 3, 10, 30, 100 mg. per litre. In the second experiment the range was 3, 10, 30, 100, 300, 600 and 1000 mg. per litre. Penicillin G did not stimulate expansion in either of these two experiments. The second experiment showed that Penicillin G was inhibitory at 300 mg. per litre and higher concentrations.

The following is a list of substances which have been shown, in the reference given beside each substance, to reversibly counteract the effect of an exogenous auxin in some plant growth systems. They are, therefore, considered to be antiauxins.

PAA = phenoxyacetic acid (McRae and Bonner, 1953)

m-MPAA = m-methyl phenoxyacetic acid (Aberg, 1956)

TCPAA = tri-chlorophenoxyacetic acid (McRae and Bonner, 1953)

o-CPIBA = o-chlorophenoxyisobutyric acid (Ditto)

p-CPIBA = ∝(p-chlorophenoxy) isobutyric acid (Ditto)

2-4-DCPIBA = ∝(2-4-dichlorophenoxy) isobutyric acid (McRae and Bonner, 1953)

TIBA = tri-iodobenzoic acid (Ditto)

MH = maleic hydrazide (Leopold, 1955)

Each of these substances was tested, over a range of concentrations, on the expansion of leaf discs light treated for 5 minutes and in the absence of cobalt. The range of concentrations in each case was 0.01, 0.1, 0.3, 1.0, 3.0, 10, 20 and 50 mg. per litre. Of these compounds o-CPIBA, p-CPIBA, 2-4-DCPIBA and MH were obtained from the California Foundation for Biochemical Research, PAA and TIBA from Eastman Organic Chemicals, m-MPAA from the Aldrich Chemical Company Incorporated and TCPAA from the Bios Laboratories Incorporated. The results are shown in Figures 21, 22 and 23. In each of these experiments a dark control and light plus cobalt control were included and showed the typical differences in final diameters.

The compounds fall into three groups. In the first group (2-4-DCPIBA and p-CPIBA) inhibition commenced at low concentrations and increased with increase in concentrations up to the highest concentration tested. In the second group (MH, m-MPAA and o-CPIBA) there was slight stimulation at low concentrations and inhibition at higher concentrations. In the case of m-MPAA the inhibition was relieved to some extent at the highest concentration. In the third group (TIBA, PAA and TCPAA) inhibition commenced at low concentrations, was relieved, to a greater or lesser extent, at higher concentrations and was inhibitory again at the highest concentrations. The most exaggerated form of this unusual curve is seen in the case of TCPAA (Figures 23 and 24) in which the inhibition by low concentrations was relieved at higher concentrations where the growth was equal to that of the light plus

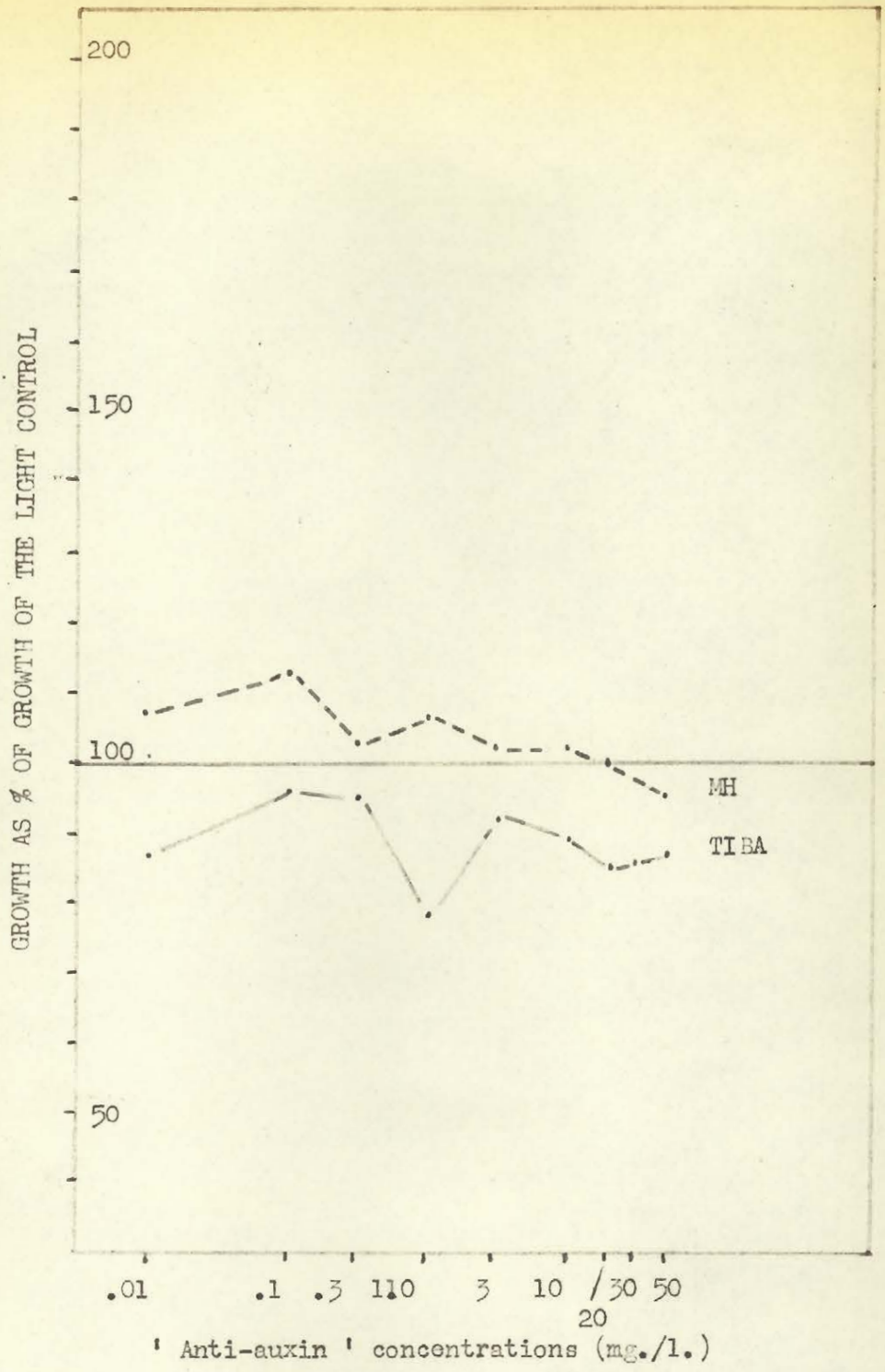


FIGURE 21. THE EFFECT OF TWO 'ANTI-AUXINS', MALEIC HYDRAZIDE (MH) AND TRIIODOBENZOIC ACID (TIBA), ON THE EXPANSION OF ETIOLATED BEAN LEAF DISCS.

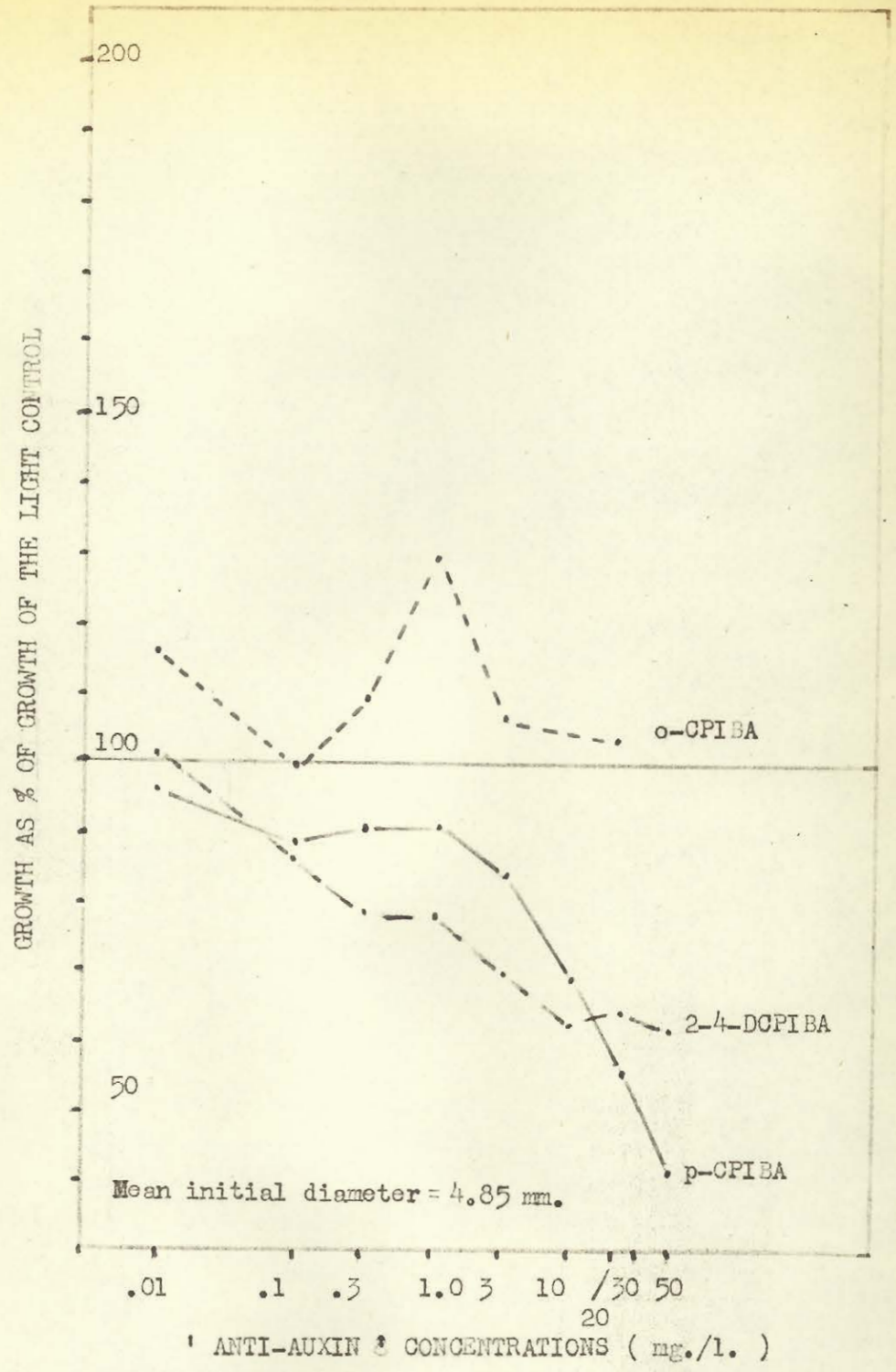


FIGURE 22. THE EFFECT OF THREE ' ANTI-AUXINS ', o-CHLORO-PHENOXYISOBUTYRIC ACID (o-CPIBA), α(2-4-DICHLOROPHENOXY) ISOBUTYRIC ACID (2-4-DCPIBA) AND α(p-CHLOROPHENOXY)ISO-BUTYRIC ACID (p-CPIBA),ON THE EXPANSION OF ETIOLATED BEAN LEAF DISCS.

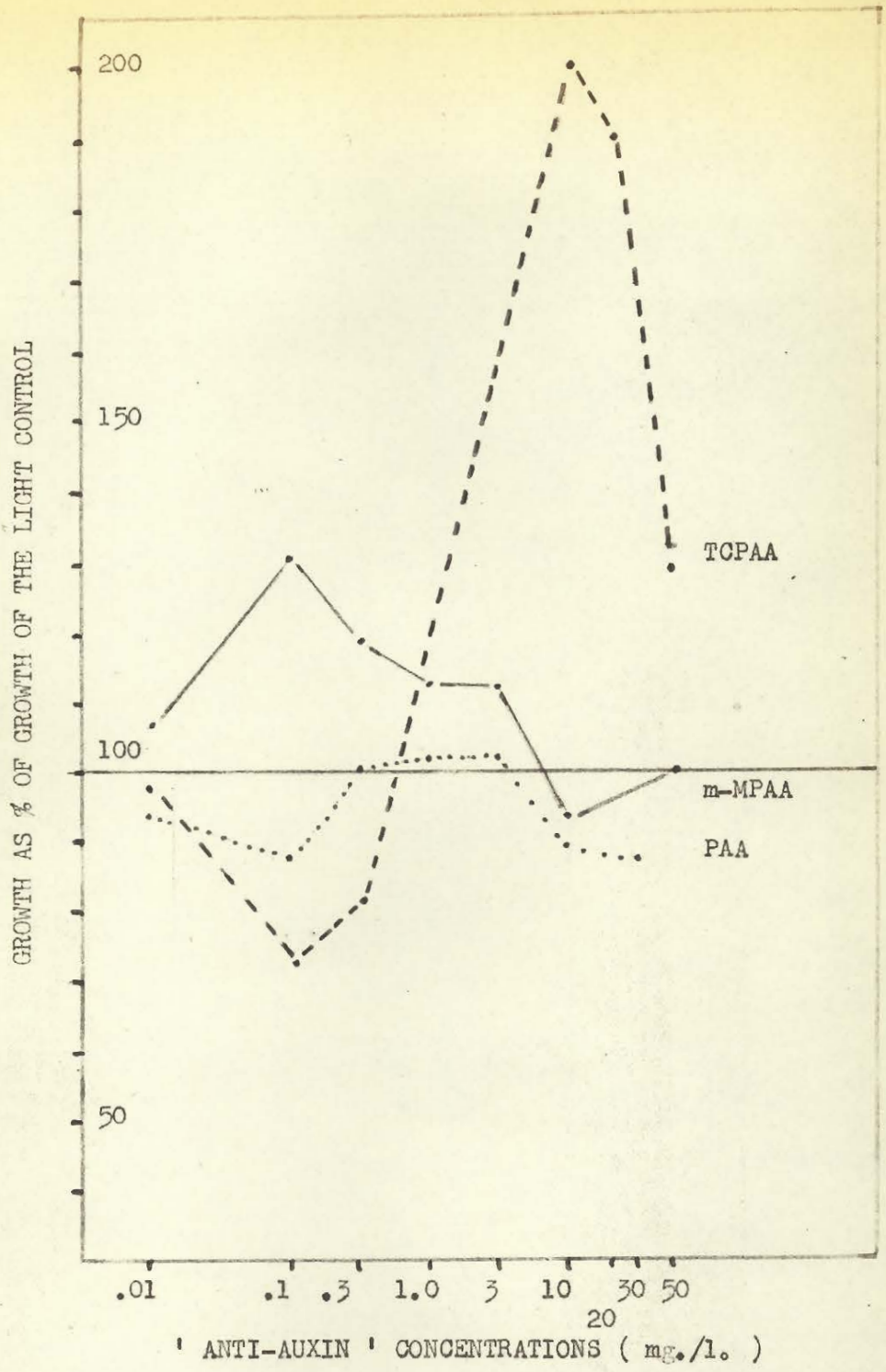


FIGURE 23. THE EFFECT OF THREE ' ANTI-AUXINS ', TRI-CHLORO-PHENOXYACETIC ACID (TCPAA), m-METHYLPHENOXYACETIC ACID (m-MPAA) AND PHENOXYACETIC ACID (PAA), ON THE EXPANSION OF ETIOLATED BEAN LEAF DISCS.

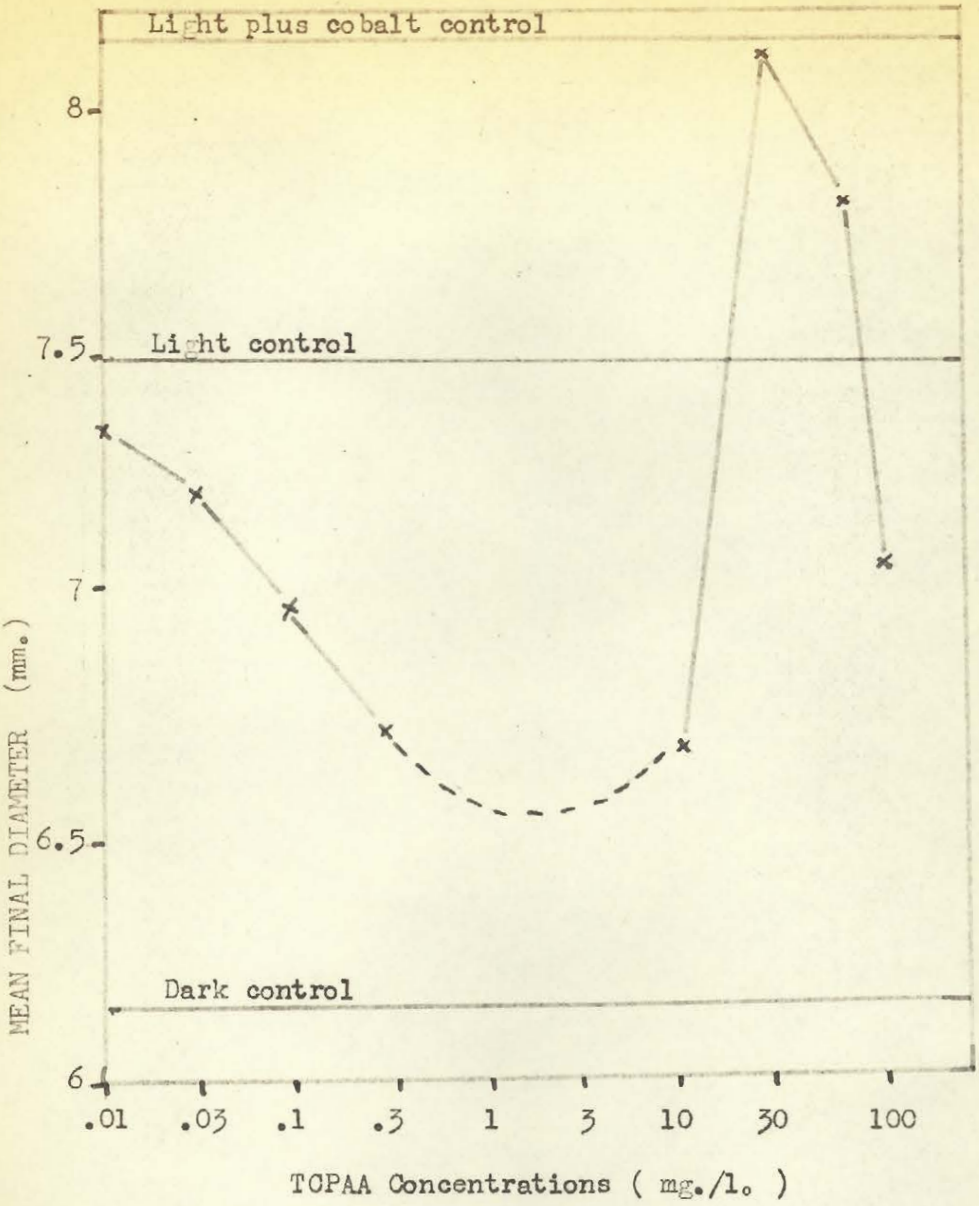


FIGURE 24. THE EFFECT OF TRI-CHLOROPHENOXYACETIC ACID (TCPAA) ON ETIOLATED BEAN LEAF DISC EXPANSION. THE MEAN INITIAL DIAMETER = 4.85 mm.

cobalt control. In other words, at appropriate concentrations, TCPAA completely replaced cobalt in the expansion of light treated leaf discs.

Some interesting incidental observations were made when the discs of this experiment were measured. These include the influences of TCPAA on (1) vein as opposed to lamina expansion and (2) the retention of yellow pigments during the experiment. After an exposure of 36 hours to light following the treatment by TCPAA, the amount of chlorophyll formed in the discs was also noted.

When the discs were measured, after 72 hours in the dark, it was noted that those treated with 0.3 to 30 mg. per litre TCPAA were curled under at the edges. This was particularly marked in the discs from the 0.3, 1 and 3 mg. per litre concentrations. It was also noted that in the discs treated with 3 to 30 mg. per litre TCPAA the thickness of the veins was considerably greater than in the other treatments, including the controls. At 100 mg. per litre and above, curling at the edge occurred but in an upward direction so that the discs looked like disc shaped boats. An inhibition in disc size accompanied this latter curling (Figure 24). The curling under of the discs in the lower concentrations and then upwards in the higher concentrations indicates that in the first case a stimulation of vein increase occurred without a corresponding stimulation of the lamina increase and in the latter case there was an inhibition of the vein increase.

The discs, at the time they were measured, were found to differ in the amount of yellow pigment present. The discs treated with 0.3 - 100 mg. per litre of TCPAA were a very pale yellow as compared to those of other treatments and the controls. This suggests that TCPAA either leads to the breakdown of the carotenoids present or inhibits the synthesis of carotenoids which are being broken down continuously in the discs.

After the discs were measured and exposed to white light for 36 hours it was noted that chlorophyll was lacking in the discs which had been previously treated with 10, 30 and 100 mg. per litre TCPAA. It would appear that pretreatment with an antiauxin, or with cobalt (Section 4.621) and ethionine (Section 4.622), prevents chlorophyll formation in leaf discs after they are exposed to white light.

The interpretation of the results presented in this and the previous section would be facilitated by information on the following: (a) the effect of methionine alone, and together with ethionine and (b) the effect of auxins on the inhibitions or stimulations obtained with certain of the antiauxins used here. Unfortunately, it was impossible to complete this series of experiments because of persistent fungal contamination of the leaf discs. Recent correspondence with Miller indicated that, after he moved to Wisconsin from Ohio State University, he encountered fungal contamination which has made the use of the test impossible at Wisconsin. It should be possible to eliminate this problem, with its attendant waste and frustration, by growing, and manipulating the bean plants under completely sterile conditions. However, it was not possible to construct the necessary facilities within the project reported here.

5.0 DISCUSSION

The use of amino acid antagonists to obtain new information about metabolic pathways in microorganisms and animals has been quite extensive (Schrank, 1956). The use of ethionine for this purpose in bacteria and rats will be discussed later. Its use to study the metabolism of higher plants was neglected until Boll (1954d) reported an inhibition in growth of excised tomato roots by ethionine. The main points from this work, which is discussed in Section 2.4, are repeated here to facilitate the general discussion. The inhibition was relieved by methionine, homocysteine, homocystine, and to some extent, ethanolamine. Boll also reported that ethionine and various ratios of methionine (or homocysteine) to ethionine caused some significant changes in the morphology of the roots. The influence of ethionine upon the morphology of these roots, particularly upon the frequency of lateral root production, was of added interest because the response to ethionine or ethionine plus methionine was influenced by the presence or absence of niacin. It is known that the frequency of lateral root production can be altered in a similar way by IAN (Street et al, 1954) and NAA (Boll, unpublished). Tryptophane is a precursor common to both niacin and IAA. Although IAA is not known to occur in tomato roots, it is possible that IAA, or some product thereof, is involved in the growth process. Thus when niacin is supplied to the roots the growth effects could be, at least in part, due to an increase in IAA synthesis as a consequence of the greater availability of tryptophane. Thus it could be the resultant

increase in auxin quantity which was influencing the response to ethionine, or ethionine plus methionine, and not the niacin molecule itself. Considering the above data, it was felt that there was strong evidence to support the idea that, in the excised tomato root, there is a relationship between the metabolism of methionine and the suspected hormonal mechanism involved in the growth of the root.

The purpose of the research reported here (Sections 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6) was to determine whether ethionine could influence the growth of plant systems, other than the isolated tomato root, which are suspected or known to involve an auxin controlled growth mechanism. If ethionine was found to exert an auxin-like influence upon plant growth systems in general, it was hoped that at least one system would prove suitable for the determination of the mode of action.

A summary of the known effects of ethionine on higher plants, including the results of the research done herein, is given in Table XIII. From this table it will be noted that high concentrations of ethionine usually inhibit growth and that a stimulation in growth occurred at relatively low ethionine concentrations (1 to 60 gamma per litre) in all but four of the eleven systems studied. In one of the four (etiolated bean leaf discs) stimulation of expansion occurred at 600 gamma per litre ethionine following a light treatment. In a second (lateral bud development in green beans) a possible stimulation occurred at 60,000 gamma per litre ethionine. This stimulation by ethionine is of doubtful nature considering the suspected presence of an oxidizing agent in the sealing agent used (see Section 4.12). It may be that, under the conditions of these experiments, much of the ethionine was destroyed or that a high external concentration was required to give a low endogenous concentration which was actually responsible for

TABLE XIII

THE EFFECT OF ETHIONINE ON SOME PLANT GROWTH SYSTEMS

TEST SYSTEM	STIMULATORY CONCENTRATION (gamma/litre)	INHIBITORY CONCENTRATION (gamma/litre)	REFERENCE
1) Systems involving cell size of excised shoot tissue			
a) Increase in length of etiolated pea epicotyl sections	10	--	Reported herein
b) Expansion of etiolated bean leaf discs	600	1,800	Ditto
c) Elongation of *Avena* coleoptile sections	low conc. not tested	10,000=50% inhibition	Schrank, 1956
d) Growth curvature of *Avena* coleoptile sections	Ditto	2,000	Ditto
2) Systems involving growth of shoot tissue (intact) as a product of cell division and cell enlargement			
a) Lateral bud development in dwarf green beans	60,000 (?)	none	Reported herein
b) Flax seedling hypocotyl growth	1-50	500	Ditto
3) Growth of intact roots			
a) Rooting of etiolated excised bean hypocotyls			
i) plus one cotyledon	60	300	Ditto
ii) plus zero cotyledons	1.8	300	Ditto
b) Growth of roots of flax seedlings	10-50	500	Ditto
c) Growth of roots of wheat seedlings (in this case it was possible to measure increase in cell size alone)	10	100	Ditto
4) Growth of excised roots as a product of cell division and cell enlargement			
a) Excised tomato roots in culture			
i) without niacin			
length of main axis	6.0	12.0	Boll, unpublished
No. of lateral roots	6.0	30.0	
length of 10 basal roots (laterals)	30.0	60.0	
ii) with niacin	12.0	30.0	
length of main axis	12.0	30.0	
No. of lateral roots	1.2	60.0	
length of 10 basal roots (laterals)			
b) Excised flax root tip growth	50	500 (50%)	Ditto

the response. Another reason for the stimulatory response at such a high concentration may be that this was the only test system in which green plant tissue was treated. The other two growth systems which were treated with ethionine, and in which stimulation by ethionine did not occur, were the elongation and growth curvature tests of Avena coleoptile sections (Schrank, 1956). However, the effects of concentrations lower than 1000 gamma per litre were not reported by Schrank. It is interesting to note that the stimulation in growth due to a stimulation in cell size increase (Table XIII - numbers 1a, 1b, 2b and 3c) was obtained by the same low ethionine concentration in three of the four plant systems involved. A synthetic auxin has been shown to influence the cell size increase in each of these systems which are 1a) elongation of etiolated pea epicotyl sections (reported herein), 1b) etiolated bean leaf disc expansion (Liverman, 1955), 2b) flax seedling hypocotyl growth (Boll, unpublished) and 3c) growth of roots of wheat seedlings (Burstrom, reported by Bentley, 1958).

Although synthetic auxins have not been shown to stimulate lateral bud development as ethionine has been shown to do here, but rather to inhibit it, they are known to stimulate lateral rooting on cuttings (Leopold, 1955) which ethionine does on the etiolated bean hypocotyls reported herein (Section 4.222). As has already been mentioned, Street et al (1954) and Boll (unpublished) have shown that IAN and NAA increase the frequency of lateral root production in isolated tomato roots. Ethionine also causes such an increase. From the above results it is evident that ethionine, to some extent, reproduces most of the effects known to be caused by auxins in a number of plant growth systems.

As well as the evidence for the stimulation of the auxin controlled growth systems by ethionine, mentioned above, there is evidence for the

interaction of ethionine either with exogenous IAA or other factors which affect auxin controlled growth processes. Thus it is shown here that auxin (IAA) or ethionine stimulated pea section growth and that there was also an interaction between ethionine and IAA at 500 gamma and 0.1 mg. per litre respectively (Section 4.52). This may mean that IAA and ethionine are active in the same process. Light (Miller, 1952) and IAA (Liverman, 1955) will stimulate the increase in the size of cells of etiolated bean leaf discs, although the stimulation due to IAA was not consistent (Liverman, 1955). In the research reported here (Section 4.622) it was shown that ethionine, following a light treatment, stimulated the growth of the cells above the stimulation due to light alone. Furthermore, it was shown that, in this light regulated growth process, there was an interaction, though complicated, between ethionine and duration of the light treatment.

In order to provide a discussion of possible relationships between ethionine and auxin action, a general discussion of the metabolic functions of methionine, the known interference sites of ethionine upon metabolism (particularly methionine metabolism), a brief summary of some theories of auxin action, and how ethionine could affect the postulated mechanisms, is given in the following sections.

METABOLIC FUNCTION AND RELATIONSHIPS OF METHIONINE

Methionine is an essential sulfur containing amino acid in metabolism. It is formed by methylation of homocysteine. The origin of the methyl group for methionine is not certain but it may come from choline or a one-carbon fragment produced from serine (Challanger, 1951; Brown and Byerrum, 1952). The formation of methionine from homocysteine is thought to be reversible

(Bonner, 1950). The metabolic functions of methionine are summarized in a word-diagram (Figure 25) and are listed below in more detail

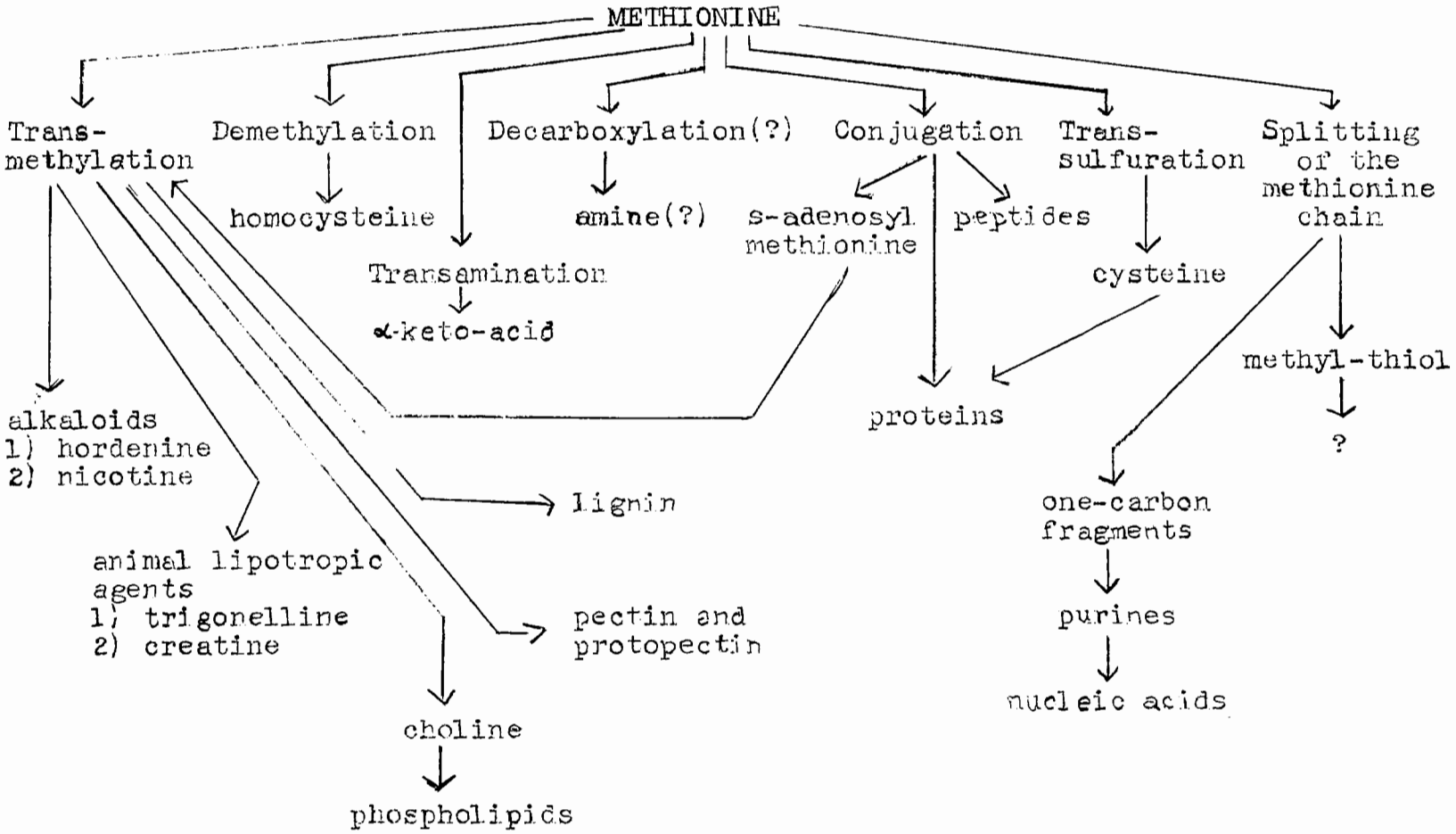
Transmethylation. Methionine has been shown to be a source of labile methyl which is used in the production of a number of methyl containing compounds in both plants and animals. 1) The incorporation of methionine methyl into plant alkaloids such as nicotine in tobacco plants (Dewey et al, 1954; and Axelrod, 1955) and into hordenine of barley (Matchett et al, 1953) has been demonstrated. 2) Methionine transmethylation has been shown to occur in mammalian tissue with the resultant formation of creatine and trigonelline (Challanger, 1951). 3) 2-methylethanolamine, 2-dimethyl-ethanolamine and choline (Matchett et al, 1953) are produced from methionine in plants by transmethylation. These compounds are important for the synthesis of phospholipids (Bonner, 1950). 4) It has also been found that the methoxyl groups of lignin can arise from the direct transfer of methyl groups from methionine (Byerrum et al, 1954) and 5) these methyl groups are also used in the synthesis of pectin and protopectin (Sato et al, 1957).

Demethylation. The removal of the methyl group from methionine results in the production of homocysteine (Bonner, 1950) which may become re-methylated to produce more methionine with its labile methyl groups.

Splitting of the methionine chain. The methionine chain may be split with the production of a) methyl thiol groups or b) the production of one-carbon fragments which may enter purines and are thus necessary for nucleic acid synthesis. With regard to the latter, Reddi (1955) has shown that methionine stimulates the incorporation of P32 into ribosenucleic acid (RNA), into adenylic, guanylic and cytidylic acids but not into uridylic acid.

Trans-sulfuration. The result of this process, which involves prior conversion

FIGURE 25. WORD DIAGRAM SHOWING THE METABOLIC PATHWAYS OF METHIONINE



to homocysteine and reaction with serine, is the production of cysteine which is an important sulfur amino acid found to be incorporated into proteins (Bonner, 1950).

Transamination. According to Meister (1955) and Singer and Kearney (1955), methionine is known to participate in transamination reactions. Presumably the corresponding keto-acid is formed although this has not been isolated.

Decarboxylation. Although the corresponding amine of methionine is not known to occur, other amino acids undergo decarboxylation to produce amines. The possibility that an amine exists corresponding to methionine cannot, therefore, be neglected.

Conjugation. Methionine is known to combine with other compounds. One case is the formation of s-adenosylmethionine in the presence of adenosine triphosphate (ATP). The adenosine-methionine complex has been shown to function as an active methyl donor in transmethylation (Cantoni, 1952; Singer and Kearney, 1955). Methionine is also a constituent of plant proteins (Bonner, 1950) and is reported to be a part of an adaptive enzyme, tryptophane peroxidase, in rats (Lee and Williams, 1952). It is likely that methionine is incorporated into peptides as well as into proteins.

THE INTERFERENCE WITH METABOLIC PATHWAYS BY ETHIONINE

Ethionine has been reported to be an inhibitor in a number of biological systems. Most of the data for these inhibitions have been obtained by research on animals and microorganisms. The exact mode of action of ethionine is still not known. The points at which it is known to inhibit metabolism are given below together with some alternative suggestions.

Ethionine inhibits transmethylation. It has been reported that ethionine prevents transmethylation by the transfer of the ethyl group to compounds which normally receive the methyl group of methionine. One example is the formation of triethylcholine in rats (Martin, 1951). On the other hand Challanger (1951) states that ethionine does not undergo transethylation. If this is true, it may be that under certain conditions the presence of the entire ethionine molecule prevents the transfer of the methyl group of methionine. There are many reports of the lipotropic action of ethionine in rats and the reversal of this interference by methionine (Farber et al, 1949; Koch-Weser et al, 1951; Farber et al, 1951; Fischer, 1955 as reported by Novelli, 1957). Although the mode of this interference is not known, it is suspected that it is due to interference with transmethylation from methionine in the synthesis of choline and creatine (Simmonds et al, 1950).

Ethionine and homocysteine formation. The prevention of homocysteine formation from methionine by ethionine has not been reported specifically. However, Farber et al (1949) showed that ethionine prevents transmethylation in rats which indicates that homocysteine production from methionine was inhibited. Stekol and Weiss (1950) showed that with ethionine labelled with C^{14} in the methyl C of the ethyl group and with S^{35}, the ethionine sulfur was available for cysteine synthesis which would tend to suggest that ethionine, as well as methionine, could be used for homocysteine synthesis.

Ethionine and cysteine formation. As has been mentioned above, Farber et al (1949) reported the inhibition of cysteine synthesis by ethionine in rats and the reversal of this inhibition by methionine. Since then, Stekol

and Weiss (1950) have shown that in rats ethionine labelled with S^{35} is used for cysteine synthesis. Thus ethionine may either prevent cysteine synthesis, or act as a precursor in cysteine synthesis.

Ethionine and transamination reactions. It has been shown that ethionine does not inhibit transamination from methionine but undergoes transamination itself (Meister, 1952; Jacquez et al, 1952, as reported by Meister, 1955). Presumably the α-keto acid derivative of ethionine is formed and this may be an inhibitory analogue of the corresponding methionine derivative. However, this has not been shown experimentally.

Ethionine and the splitting of the methionine molecule. There is no evidence to suggest that ethionine interferes with the splitting of the methionine molecule in the formation of methylthiol. However, Smith (1954), has reported that ethionine has been shown by Swenseid and coworkers to prevent the enzymatic production of one-carbon units in rats. According to Smith it is a substance enzymatically produced from ethionine which is the inhibitor. It could be that these one-carbon units are those, normally produced from methionine, which are necessary for nucleic acid synthesis. If so, this is a possible point at which ethionine might interfere with nucleic acid synthesis.

Ethionine and anthocyanin formation. Both ethionine and methionine will inhibit anthocyanin formation in Lemna minor (Thimann and Radner, 1955). There are two possible reasons for these inhibitions. The ethionine may be converted to methionine thus producing a toxic quantity of methionine. On the other hand it is possible that some part of the ethionine and methionine molecule, which is common to them both, may be the cause of the inhibition. In other words, inhibitory effects of ethionine may, in

some cases, be due to the conversion of ethionine to a product which is normally formed from methionine.

Ethionine and s-adenosylmethionine formation. In transmethylation, methionine first reacts with ATP to form an adenine sulfonium compound s-adenosylmethionine (Borsook and Dubnoff, 1947). Schlenk and Tillotson (1954) showed that 5-ethylthioadenosine was synthesized by yeast in ethionine enriched cultures and Axelrod (1955) reported that, in the reaction of methionine with ATP, ethionine can be substituted for methionine yielding ethylthioadenosine. In a very recent paper (Parks, 1958) it was shown that s-adenosylethionine was formed in yeast cultured in an ethionine enriched medium and transethylation between this substance and homocysteine was demonstrated in an in vitro system. Ethionine suppressed the formation of s-adenosylmethionine and there was some evidence for a competitive interaction between the ethyl- and methyl- donors. Presumably the s-adenosylethionine leads to the synthesis of a variety of abnormal ethyl derivatives which lead to a failure in normal processes in the cells.

Ethionine and protein formation. Considerable information exists which shows that ethionine interferes with protein synthesis. The information is obtained from two lines of evidence a) experiments with radioactive tracers and b) studies on the formation of adaptive enzymes. The interference may occur in one of two ways.

The following data suggest that the presence of the entire molecule prevents the incorporation of amino acids into proteins. Farber et al (1949) reported that in rats ethionine inhibited the uptake of glycine in vitro and in vivo and it also prevented the uptake of methionine labelled with S^{35}. Excess methionine relieved this inhibition of protein synthesis.

Similar inhibitions are reported for mouse liver by Simpson et al (1950). Wu and Bollman (1954) have also reported the inhibition of the incorporation of methionine into rat protein.

Other evidence shows that ethionine prevents the incorporation of methionine into protein by substituting for it in the protein. Levine and Tarver (1951) working with rats, and Kamin and Handler (1957) reviewing the work of Gross and Tarver with Tetrahymena, have reported that ethionine labelled with C^{14} was incorporated into the protein molecule in place of methionine. According to them this substitution of ethionine for methionine produces an abnormal protein which is consequently "sterile".

Opposing the above reports of ethionine interference with protein synthesis are other reports reviewed by Stekol (1957) which state that ethionine does not interfere with protein synthesis. Despite these reports, there is a second line of evidence, involving the study of the formation of adaptive enzymes, which suggests that ethionine does interfere with protein synthesis. An adaptive enzyme, as opposed to a constitutive enzyme, is one which is only formed, or active, in the presence of its substrate (Wagner and Mitchell, 1955). The existence of an adaptive enzyme is indicated by a lag phase in either the growth of the organism, or in the development of enzyme activity. If the formation of an adaptive enzyme is inhibited by ethionine, as some are reported to be (see below), the inhibition is presumed to be due to the prevention of the formation of the protein part of the enzyme. There are two reports of ethionine inhibiting the production of adaptive enzymes. Firstly, in rat liver, the formation of the adaptive enzyme tryptophane peroxidase has been shown to be inhibited by ethionine (Lee and Williams, 1952). Secondly, Barrett et al (1953) reported that fumarate grown cells of Pseudomonas showed a distinct lag phase when

transferred to a citric acid medium. This was due to a lag in the formation of citric acid oxidase which is considered to be an adaptive enzyme. The lag phase was prolonged in the presence of ethionine but this inhibition of the formation of the enzyme was overcome by methionine. These two reports add support to the tracer evidence which shows that ethionine interferes with protein synthesis and with the incorporation of methionine into proteins.

POSSIBLE REASONS FOR THE STIMULATION OF PLANT GROWTH BY ETHIONINE

In view of the point, as shown in the preceding section, that ethionine might affect metabolism in a variety of ways, it is not possible to provide a simple explanation of the stimulation of growth by ethionine. Some of the more interesting possibilities are given below and particular attention is given to the possibility that the stimulation is through an effect on the auxin controlled growth mechanism.

Ethionine may be exerting its stimulatory influence upon the growth of plant systems in a number of ways. (1) It may act by blocking an inhibition of a metabolic pathway caused by the presence of an excess of methionine. It is known that amino acids, although they are metabolites, will act as antimetabolites (see Tables V and VI). Martin (1951) has suggested that naturally occurring antimetabolites may be responsible for regulating the amount of a metabolite which is free to take part in metabolic pathways at any one time. Ethionine may be exerting its influence in this way. The possibility that either an ethyl analogue or an ethionine-containing analogue of some metabolic product of methionine might prevent an inhibition due to excess methionine is suggested by the work of Parks (1958) mentioned above. Parks' results indicated a competitive inter-

action between S-adenosylmethionine and S-adenosylethionine. 2) The stimulation may be due to a product common to both ethionine and methionine and need not be due to the antimetabolite properties of ethionine as such. 3) Ethionine may exert its influence through becoming de-ethylated and then methylated to produce more methionine which would then be available for one of a number of syntheses which are summarized in Figure 25.

Some of metabolic pathways of methionine, particulary those involving transmethylation to produce phospholipids, are probably very important for growth. Phospholipids are believed to be components of the cell membrane which is the suggested site of transport of various substances including growth regulators. Current views on the growth of cells include the idea that an intimate connection between surface layers of the cytoplasm and the cellulose structure of the walls is necessary for the growth of the walls (Frey-Wyssling, 1952). The phospholipids may be of critical importance at this point. Furthermore, an increase in nucleic acids or proteins, which have many functions in biological systems, could exert a favourable influence upon the growth mechanism of plants.

In order to show how ethionine might exert its influence on plant growth via an auxin controlled growth mechanism, rather than through an influence on metabolism in general, a brief summary of some of the theories of auxin action is presented in the next section.

POSSIBLE RELATIONSHIPS OF ETHIONINE WITH AUXIN ACTION

Numerous theories exist with regard to auxin action and are reviewed by Bonner and Bandurski (1952), van Overbeek (1952), Audus (1953), Leopold (1955), Aberg (1957) and Bentley (1958). Recent work has brought about the

general conclusion that the effect of auxin on the regulation of growth is primarily on the cell wall and the regulation, or the alteration, of plasticity. According to Bentley (1958), this is a return to the early conclusions of Heyn (1940). A few theories of auxin action, and the possibility of ethionine influencing the postulated mechanism, will be discussed below.

A close connection between auxins and nucleic acid metabolism has been established through the work of Skoog et al (1942) from studies on the effect of IAA on nucleic acid levels in tissues of tobacco pith in culture, corn roots and lateral buds of Tradescantia. They concluded that marked IAA-induced increases in ribose nucleic acid (RNA) are associated with cell enlargement and marked increases in desoxyribose nucleic acid (DNA) with cell division. They propose that the auxin level affects the DNA/RNA ratio, which in turn influences rates of cell multiplication and cell enlargement. It has been shown that the purine adenine (or adenosine) is implicated in the following growth systems; (1) leaf development (Bonner and Haagen-Smit, 1939), (2) growth and bud development in tobacco stem segments (Skoog and Tsui, 1948), (3) growth of epicotyl sections, leaf buds and roots of etiolated beans (Galston and Hand, 1949) and (4) morphogenesis of Lemna minor (Hillman, 1955).

If this theory of auxin action is correct, and assuming that excess auxin leads to excess nucleic acid synthesis, then the stimulation of growth by ethionine could be explained in two ways depending upon whether a particular growth system, stimulated by ethionine, contains a supra-optimal or a sub-optimal concentration of endogenous auxin. With supra-optimal auxin, ethionine could stimulate growth by interfering with the

production of the one-carbon fragments from methionine which are necessary for purine synthesis. On the other hand, with sub-optimal auxin, and where purine synthesis was limited by methionine, ethionine could stimulate growth if, as suggested previously, it replaced methionine as the source of one-carbon fragments. It is, perhaps, significant in this connection that the purine analogue kinetin will stimulate the growth of etiolated bean leaf discs (Miller, 1956). The experiments done here do not permit any choice between these alternatives but some simple nutritional experiments in which either methionine or purines are supplied in place of ethionine, or in combination with ethionine, could indicate whether ethionine merely functions as a source of products which normally arise from methionine.

It has been suggested that IAA might act through a chelation mechanism in removing calcium from the cell walls (Bennet-Clark, 1956). Inter-relationships have been shown between IAA and calcium in root cell elongation (Burstrom, 1956 and 1957). The chelating compound ethylenediaminetetra acetic acid (EDTA) has been found to influence the elongation of coleoptiles (Heath and Clark, 1956a and 1956b) and lupin hypocotyls Weinstein et al, 1956). These people showed that a number of substances with chelating properties, but no structural resemblance to auxins, showed auxin activity. EDTA was one of the most active of these chelating compounds. In the work reported here EDTA had no effect upon the expansion of the etiolated discs which suggests that, in this system at least, the stimulatory effect of ethionine was not due to a chelating mechanism.

Numerous studies have been made of the effects of auxins on the activity of particular enzymes. The significance of the results, in regard to the biological effects of auxins, is open to question and in

many instances, the results obtained may be attributed to a non-specific physical effect of auxin on the enzyme (Leopold, 1955). However, one series of observations is significant to this discussion. Neely et al (1950) found that 2,4-D stimulated pectin methyl esterase activity in leaves and proliferating stem tissue of the red kidney bean. Similarly, Waygood et al (1954) found that auxins stimulated pectin esterase activity of the fungus Cladosporium herbarum. Perhaps the most significant results are those given by Bryan and Newcombe (1954) in one of a series of studies on the biochemical changes induced by IAA in tobacco pith cells cultured on agar medium. The cells are increased in size by IAA but cell division does not take place. Estimates of pectin methyl esterase activity, following treatment with IAA, showed that the activity increased prior to the enlargement of the cells and, during the period of rapid cell enlargement, the activity was double that of the controls.

In discussing these results, Bryan and Newcombe quote other work which supports the view that auxin causes a plasticizing of the primary wall during the early phases of cell enlargement, and that changes in the pectic substances are important in this plasticizing process. It is suggested that auxin, by stimulating pectin methyl esterase activity, initiates the breakdown of protopectins leading to a reduced tensile strength of the young primary wall and a subsequent stretching by the turgor pressure of the cell. Furthermore, there is evidence that polygalacturonase, which hydrolyzes pectin and pectic acid to free galacturonic acid, acts more rapidly on the demethylated chains than upon the partly methylated chains of pectin. If this view of auxin action is correct then the auxin-like action of ethionine could be explained by assuming that it

prevents the transfer of methyl groups from methionine to the pectin chains. The proportion of demethylated chains would then increase as a consequence of continued pectin methyl esterase activity and, in turn, these chains would be broken down by polygalacturonase.

Another theory of auxin action is based upon the relation between the structure and activity of growth regulators. According to Skoog et al (1942) auxin could be acting as a protein prosthetic group of an enzyme system which influences the growth process. In order for this to occur the auxin molecule must have two fundamental properties - (1) the necessary structural configuration to combine in some manner with the protein portion of the enzyme and (2) a specific group which reacts with the substrate molecule. An argument in support of this theory is given by Skoog (1947) who states that auxin controlled reactions are inhibited by the presence of growth-inhibitors with an auxin-like structure which possesses, strongly, only one of the two properties listed above, and the other property only weakly or not at all. Meyer and Anderson (1952) explain that if such an inhibitor possesses only the capacity of combining withthe protein, it may act as an inhibitor by occupying positions on the protein complex which would otherwise be taken by more active auxins. On the other hand, if the auxin-like compound possesses only the property of reacting with the substrate, it may block the overall reaction which can only take place if the compound can act as a chemical bridge between the protein and the substrate molecules. It has been suggested, in the two-point attachment theory, that in the growth reaction two parts of the auxin molecule, the acidic side chain and the unsaturated ring nucleus, may each react with some proteinaceous materials (Hansch et al, 1951; McRae and Bonner, 1952). Support for the

theory that auxin is attached to protein is available in that 1) free auxin is released from plant tissues and plant proteins by proteolytic agents; 2) labelled synthetic auxins quickly find their way into substances sedimented by typical protein precipitants and 3) the kinetics of auxin action in Avena coleoptiles are best understood in terms of the attachment of auxin at two points to some acceptor, (Foster et al, 1952; Foster and Bonner, 1956) presumably protein (Siegel and Galston, 1953). Despite the indicative nature of these data, the "auxin-protein" has remained hypothetical rather than a reality.

Since Hammetts' (1930) early observations on cell growth, many studies have indicated a close connection between sulfhydryl (SH) metabolism and plant growth processes (see Thimann, 1949). Some growth regulators (antiauxins) are known to combine with SH. According to van Overbeek et al (1955) and Liverman (1955), TIBA and the maleimides are examples. Added to this, there is evidence for the stimulation of growth by cobalt which is known to form reversible complexes with cysteinyl-SH (Michaelis and Yamaguchi, 1929; Michaelis, 1929). Miller (1952) first reported the stimulation of leaf discs by cobalt and since then Liverman (1955), and the research reported herein (Section 4.621), have verified Miller's results. Thimann (1956) has reported that cobalt stimulates pea section growth. Although it has been shown that auxin is involved in the growth mechanism of the pea sections (Section 4.52), Liverman (1955) states that there is no consistent stimulation of leaf disc growth by auxin. The stimulation of leaf expansion due to certain antiauxins (Section 4.623), particularly TCPAA (Figure 24), suggests strongly that auxin is involved in the growth system too, but may generally be present in supra-optimal concentration.

The failure of the anti-auxin TIBA (a known SH reactant) to stimulate growth of the discs (Section 4.623) may be due to its structural incompatibility with the specific, auxin-controlled growth mechanism in the leaf disc. Thus it is not felt that the lack of TIBA stimulation is strong evidence for the argument that there is no hormonal mechanism operating in these discs.

Considering the above data it is not surprising that several studies have led to suggestions of various schemes which involve protein SH in the mechanism of auxin action (Thimann, 1949; Siegel and Galston, 1953; Leopold and Guernsey, 1953; Veldstra, 1953; Leopold, 1955; Muir and Hansch, 1955; Tonzig and Marre, 1955; van Overbeek et al, 1955; Bonner and Foster, 1956). Among these studies have been attempts to find a protein substance to which auxin attaches at an SH group.

In an attempt to find the SH containing substance to which auxin is attached Siegel and Galston (1953) have shown that IAA is incorporated into pea root proteins in vivo by an energy requiring process and in vitro by the use of adenosine triphosphate (ATP). Measurements were made showing the disappearance of the SH groups in the presence of the auxin. Coenzyme A (CoA) was shown to remove IAA from the protein in vitro. The authors suggest that the reaction between CoA and IAA-protein involves thioether formation, although Leopold and Price (1956) have since rejected this involvement of thioether formation. Leopold and Guernsey (1953) have shown that many auxins can react with CoA, and that several characteristics of the reaction suggest that CoA is concerned with the process of plant growth. Muir and Hansch (1955) have suggested that auxin acts by combining with the cysteinyl-SH of the protein but according to Price and

Leopold (1957) direct evidence for such a reaction has never been proven. It may be that auxin is combining with the cysteinyl-SH of CoA in the scheme of Seigel and Galston.

In the work reported here it is shown that growth of etiolated bean leaf discs was stimulated by ethionine following a light treatment. Some attempts were made to determine whether substances believed to react with SH groups, and involved in growth, would also stimulate growth of the discs following a light treatment. Sulfhydryl inhibitors such as lewisite, iodoacetate or methyl bromide were not included in the studies because their effects could be general effects on metabolism rather than on the SH of the growth process. Stimulation by cobalt, as reported by Miller (1952) and Liverman (1955) was confirmed. Penicillin was inactive as was tri-iodo-benzoic acid (TIBA) and some other "antiauxins". However, the "antiauxin" trichlorophenoxyacetic acid (TCPAA), at certain concentrations, caused a marked increase in growth equal to, or greater than, that obtained with cobalt. There is, of course, no evidence, other than by analogy with other "antiauxins", that TCPAA reacts with SH groups. The failure of TIBA to stimulate growth may be due to its structural incompatibility with the specific auxin controlled growth mechanism in the leaf disc. Thus the results of these experiments, as is so frequently the case with growth experiments of this type, do not provide any clear correlation between growth stimulation and the believed SH reacting properties of the applied substances. It can be said that the three substances cobalt, ethionine and TCPAA, which may affect sulfhydryl groups, will stimulate growth of the discs.

The SH groups of proteins are, as far as is known, part of the cysteine molecules which can be recovered from proteins. The only other sulfur amino

acid known to occur in proteins is methionine. Presumably the methionine residues could yield free -SH groups if the methionine is demethylated *in situ*. This possibility is worth considering although there is no evidence that it occurs. Homocysteine has not, to my knowledge, been recovered from protein. However, many of the techniques used in protein analysis might lead to destruction of homocysteine. Furthermore, the particular protein involved in the growth reaction would probably be only a very small part of the total protein. In the plant, methionine is believed to be synthesized by a pathway which includes cysteine as a precursor. Nevertheless, methionine can be converted to cysteine and the amount of cysteine available for protein synthesis could be affected by the rate of conversion of methionine to cysteine. Thus, on the basis of points raised in this discussion, the following general explanations involving an effect of ethionine on SH-protein can be made.

1) Ethionine prevents the synthesis of cysteine from methionine and thus the synthesis of protein.

2) Ethionine prevents the uptake of methionine into protein with a consequent reduction in protein synthesis.

3) Ethionine replaces methionine in the SH protein and renders it inactive.

4) Ethionine replaces methionine in the protein and the ethyl groups are not removed to yield free SH groups.

It has been shown above, in discussing the known effects of ethionine on metabolic pathways, that ethionine, in some biological systems, can interfere with amino acid uptake into proteins, will substitute for methionine in proteins, and does interfere with the synthesis of cysteine from methionine.

It appears, therefore, that there is good reason to believe that ethionine can exert its influence upon plant growth by interfering with the availability, to auxin, of an appropriate protein molecule with a specific SH site.

In concluding this discussion of the stimulation of growth by ethionine, one further explanation should be mentioned. The level of available auxins in tissue must be controlled by the balance between the rates of synthesis and release from bound form, and the rates at which auxins are destroyed or conjugated into inactive forms. Our knowledge of pathways by which auxins are destroyed is scant. Furthermore, the number of natural auxins is uncertain and recent techniques of extraction and chromatographic separation indicate that a number of auxins remain to be characterized. However, in the case of IAA, there is good evidence for the existence of an enzyme system, IAA oxidase, which destroys IAA (Tang and Bonner, 1947; Wagenknecht and Burris, 1950; Gortner and Kent, 1953; Jensen, 1955; Pilet and Galston, 1955). One feature of this enzyme, which is particularly relevent to this thesis, is that IAA oxidase may be an adaptive enzyme (Galston and Dalberg, 1954) and it is suggested that this characteristic may explain many developmental and morphogenetic relationships of IAA (Galston, 1955). In view of the known inhibitory effect of ethionine on the synthesis of adaptive enzymes (see above) it is possible that the auxin-like action and morphogenetic effects of ethionine are simply due to ethionine inhibiting the synthesis of IAA oxidase, or some other adaptive auxin oxidase, and thus raising the effective level of endogenous auxin. Schrank's (1956) failure to record any stimulation is not inconsistent with this view. If the suggestion should prove correct, ethionine might prove a valuable tool

in physiological experiments in which it is desirable to eliminate the destruction of auxin.

Possible ways in which the research reported here might be profitably continued are discussed in the following paragraphs.

One of the objects of the present work was to attempt to obtain a plant growth system with which stimulation by ethionine is sufficiently great to permit the study of interactions between ethionine and auxins or antiauxins. Such studies could provide better evidence as to whether ethionine is directly affecting the auxin controlled growth or otherwise. Although growth stimulation by ethionine was shown here, none of the systems were stimulated to any marked degree. It is possible that the etiolated bean leaf disc could be used for the study of interaction effects but the susceptibility of the disc to fungal contamination is a serious disadvantage. Perhaps a completely sterile technique could be devised. However, it would probably be preferable to make further studies of the effect of ethionine on growth of pea sections. The studies with pea sections reported here were not definitive mainly because the facilities were inadequate and some unknown factor in the growth room was inhibiting growth, causing abnormal seedlings, and it is believed, causing a great deal of the variability which was experienced. It is felt that, following improvement of the facilities, the whole question of the effect of ethionine on pea section growth should be re-examined.

In the event that ethionine does, under appropriate conditions, markedly, and consistently, stimulate growth of pea sections, and shows an additive effect with IAA, then further experiments should be done to determine the reason for the effect of ethionine. The approaches are

suggested in, or by, the preceding discussion. Perhaps the first point, and perhaps the simplest, to examine would be whether ethionine is merely replacing methionine as a source of one-carbon fragments for purine synthesis. As suggested above, nutritional experiments involving the addition of methionine or purines alone, together and in combination with ethionine could provide some clues as to identity, or otherwise, of the action of IAA, methionine or ethionine in the growth system. These experiments could be combined with biochemical techniques. Both C^{14}-methionine and C^{14}-ethionine are available commercially and the incorporation of C^{14} from ethionine into purines could be checked. Tracer, or standard techniques, could be used to determine whether ethionine leads to an increase in nucleic acid synthesis.

If these experiments fail to give a positive result then other possibilities could be examined. The most satisfactory experiments would probably be of a biochemical nature and would compare tissues grown with or without stimulatory concentrations of ethionine.

The fate of the methyl group of methionine could be checked, using methionine labelled in the methyl group, followed by a study of the effect of ethionine on the various transmethylations. Similarly, using ethionine labelled in the ethyl group, any compounds in which the ethyl group is substituted for methyl could be isolated and identified. Particular attention should be given to the possible occurrence of S-adenosylethionine in plant tissues fed with ethionine. The biological activity of this substance, or any other ethyl analogues produced in the tissue, could then be tested providing they are water soluble and able to enter plant cells. Similarly the biological effects of any such analogue and ethionine itself,

providing the activity of the analogue is greater than that of ethionine, could be good evidence in favour of the view that the growth stimulation and morphogenetic effects due to ethionine are through an effect on transmethylation.

The effect of ethionine on protein synthesis and on sulfhydryl groups could be examined but should receive less attention because of the technical difficulties. It is very probable that, if the effect of ethionine is through an effect on protein, it will be a particular protein which may represent only a small fraction of the total protein and, therefore, will be difficult to study. Similar difficulties as well as problems of estimation (see Leopold and Price, 1957), could arise in the study of sulfhydryl groups.

The effect of ethionine on the formation of the IAA oxidase system would be a relatively simple study and well worth investigating from the point of view of both the problem discussed here and its possible value for the experimental control of auxin destruction.

6.0 SUMMARY

The research reported in this thesis was undertaken in an attempt to determine the effect, if any, of ethionine on some plant growth systems known or presumed to involve auxin. It was based on the observation that ethionine, an antimetabolite to methionine, exerted stimulatory and morphogenetic effects on isolated tomato roots grown in sterile culture (Boll, unpublished). The same observations suggested that methionine metabolism was involved with the hormonal mechanism in these roots.

The literature reviewed up until January 1, 1958 shows that antimetabolites can be used to reveal information about biochemical and physiological processes in higher plants.

The effect of ethionine was tested on seven plant growth systems of three kinds. These included:

(1) systems involving increase in cell size of excised shoot tissue,
    a) increase in length of etiolated pea epicotyl sections
    b) increase in diameter of etiolated bean leaf discs
(2) systems involving growth of shoot tissue (intact) as a product of cell division and cell enlargement
    a) development of lateral buds on green, bean seedlings
    b) growth of hypocotyls of flax seedlings
(3) systems involving growth of intact roots
    a) rooting of etiolated, excised bean hypocotyls
    b) growth of roots of flax seedlings

c) growth of roots of wheat seedlings, and in this case, it was possible to measure increase in cell size alone.

One of these tests, the rooting of excised etiolated bean hypocotyls, is a rooting test devised specifically for the work reported here. This test can be performed in the laboratory, does not require external nutrients, and the response to exogenous IAA is quantitative. It is believed that this should prove of general value.

Ethionine was shown to cause a statistically significant increase in growth in three of the systems namely, the expansion of etiolated bean leaf discs, the rooting of excised, etiolated bean hypocotyls, and the size of epidermal cells of wheat roots. The effect on flax seedlings was not consistent but in most experiments growth of the hypocotyl and root was stimulated and ethionine stimulated growth of excised flax root tips. With sections of etiolated pea epicotyls the response to IAA varied considerably. Ethionine stimulated growth in each experiment where response to IAA was high. On the other hand ethionine reduced the increase in response to IAA obtained with manganese pretreated sections. Ethionine was shown to interact with various treatments believed to affect auxin controlled growth.

Penicillin, triiodobenzoic acid (TIBA) and other "antiauxins" which might react with sulfhydryl groups did not stimulate growth of etiolated bean leaf discs. Growth of the discs was however markedly increased by trichlorophenoxyacetic acid (TCPAA).

The metabolic function of methionine and its possible relation to auxin action is reviewed. The growth stimulation by ethionine is discussed in relation to the metabolic function of methionine and theories of auxin action with particular reference to the role of sulfhydryl groups in growth.

Some incidental observations which were made during the course of this work and may be of general interest are listed below.

(1) Cobalt, or ethionine, or TCPAA inhibited the synthesis of chlorophyll in etiolated bean leaf discs transferred to light.

(2) TCPAA either led to the breakdown of the carotenoids which were present or inhibited the synthesis of carotenoids which are being broken down continuously in the etiolated bean leaf discs.

(3) The stimulation of the expansion of the lamina and veins of etiolated bean leaf discs, by TCPAA required different concentrations. As a consequence, the final morphology of the discs varied with concentrations of TCPAA.

(4) The presence of cotyledons was necessary for the production of chlorophyll in the hypocotyl and leaves of etiolated bean hypocotyls following a light treatment.

(5) The presence of leaves as well as cotyledons on etiolated bean hypocotyls permitted hypocotyl length increase in an auxin concentration which was inhibitory when cotyledons alone were present.

(6) The presence of leaves inhibited the formation of long and branched lateral roots on etiolated bean hypocotyls which were produced when the cotyledons only were present.

Possible ways in which the research reported here might be profitably continued are discussed.

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a = Bentley, 1958.

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c = Galston, 1956.

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