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Inhibition of Protein Synthesis and of Auxin-Induced Growth by Chloramphenicol [1]

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Introduction

The mode of action of auxin in causing cell enlargement has proven elusive for many years. It is generally accepted that the last phase is a change in the properties of the cell wall which allows it to become extended by osmotic forces with which it was previously in balance. This process usually follows only after a time lag, which may last several minutes or days, depending on the tissue (7, 19, 28). The preceding phases are evidently chemical in nature (see e.g. 28) but evidence about them is limited to the requirement for oxidative energy, and the participation of organic acid metabolism and of one or more sulfhydryl enzymes (see 1, 33). Much of this evidence rests on the effects of inhibitors, which have indicated that a group or chain of chemical events precedes the effects on the cell wall. Since most of the inhibitors act more powerfully on cell enlargement itself than on oxygen consumption, it is probable that the connection between growth and respiration may not be direct. The fact that auxin may in some circumstances stimulate growth without any measurable increase in oxygen consumption points in the same direction (see 33).

In slices of potato and artichoke tubers, the evidence strongly suggests a relationship between cell enlargement and the synthesis of protein (34). Although this has not been borne out in studies on other tissues, at least to the extent that auxin treatment does not always appear to increase the protein synthesis (see 21), it remains possible that there is some connection between growth and protein synthesis. Most of the data, indeed, are consistent with the possibility that auxin promotes directly or indirectly either the synthesis or the turnover of one or more special proteins concerned with cell enlargement. One possible explanation of the differences between the responses of different plant materials is that these proteins may be present in only small amounts in some tissues, but in easily measurable quantities in others.

The present study examines the possibility of a relationship between protein synthesis and cell enlargement, using C^{14}-amino acids and the inhibitor chloramphenicol. In bacteria the action of this antibiotic is specifically exerted on protein synthesis (6), and it has the same action on subcellular particles from plants (10, 11, 18, 27); there is good evidence that it inhibits protein synthesis in plant tissues also (8, 9, 10, 16, 38), although higher concentrations seem to be needed than in particulates or bacteria. Several earlier workers have applied chloramphenicol to excised plant tissues treated with auxin, but either found no inhibition of growth, or else did not ascribe the observed inhibition to the participation of protein synthesis in growth (see 21). Preliminary reports of this work have been presented earlier (20, 21), and Key (13) has recently reported comparable results with inhibitors using 2,4-dichlorophenoxyacetic acid

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as the auxin and soybean hypocotyls as experimental material.

Experiments with other inhibitors of protein synthesis and also with inhibitors of RNA synthesis will be reported in another paper. They bear out the conclusion drawn here that auxin-induced cell enlargement is dependent on continual protein synthesis.

Materials and Methods

Materials. Unhusked oat seeds, *Avena sativa* var. Victory, were soaked in water for 4 hours and germinated in vermiculite. The seedlings were kept in darkness at 25° and were exposed to red light for 12 hours on the third day. The coleoptiles, about 3 cm long, were harvested at about 92 hours after the start of the soaking; the primary leaves were removed and, after floating on distilled water for 1 and one-half to 2 hours, 10-mm sections were taken beginning 2 to 3 mm below the tip.

Pea seeds, *Pisum sativum* var. Alaska, were soaked in water for 6 hours and laid between moist paper towels for 48 hours. The seedlings were then transferred to a rack with their roots dipping into water and grown in darkness, with occasional dim red light, for 7 days, at 25°. Twenty-mm sections were taken from the third internode starting 1 mm below the hook. The preparation of both oat and pea sections was carried out under low-intensity red light.

Artichoke tubers, *Helianthus tuberosus*, were obtained from a supplier in Oregon and grown locally. They were stored in moist sand at 3°, and disks 1×10.5 mm were prepared from sprouting tubers according to the methods given by Thimann and Loos (34). The disks were allowed to soak in a thin layer of water (about 1 mm deep) for 24 hours before use. At this time these "aged" disks weighed about 90 mg apiece.

Treatments. The incubation medium used in the experiments on oat coleoptile sections contained 2 % sucrose, 0.1 mM penicillin GK and 5 mM potassium phosphate buffer at pH 5.5, with or without auxin, chloramphenicol and C^{14}-amino acids. The coleoptile sections were incubated on a rotary shaker. The etiolated pea stem sections and the artichoke tuber disks were treated with solutions containing only auxin, chloramphenicol and C^{14}-amino acids. The pea stem sections were floated on the solutions in a petri dish; the artichoke tuber disks were supported on a plastic screen so as just to break the surface of the solution. The growth studies were performed with 10 sections per sample and 4.0, 20.0 and 25.0 ml of treatment solution for the coleoptile, stem and tuber tissue respectively. For experiments of 24 hours' duration, 10^{-4} M crystalline penicillin G was usually added. In the experiments with C^{14}-amino acids, each sample consisted of 50 coleoptile, 20 pea stem or 10 tuber sections incubated with 20, 20 and 25 ml of solution, respectively. All incubations were carried out at 25° in darkness with occasional dim red light.

The C^{14}-amino acids were in all cases supplied for 5 hours, this being the first 5 hours of treatment for coleoptile and stem sections, and the 5 hours beginning 24 hours after placing in solution for the tuber disks. The coleoptile and stem sections received 0.25 and 0.50 μc of C^{14}-amino acid, respectively in 20 ml of solution, the tuber disks 0.62 μc in 25 ml. Since these concentrations correspond to about 4×10^{-6} M and the incubation lasted only 5 hours at 25°, growth of bacteria was not a problem. In any case antibiotic was present in most experiments.

In the preincubation experiments of table VII, coleoptile sections were incubated on a rotary shaker in 0.50 μc of C^{14}-leucine for 2 hours. After washing, the sections were incubated with or without chloramphenicol and IAA for 4 hours without rotary shaking.

The auxins used were 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthalene acetic acid (NAA), and indole-3-acetic acid (IAA), diluted from stock solutions containing 100 mg per liter, adjusted to pH 5.5 with NaOH. The labelled amino acids were L-leucine-1-C^{14}, specific activity 6.5 mc per mmole, and L-proline-UL-C^{14}, specific activity 4.9 mc per mmole.

Fractionation and Isolation of the Proteins. After careful washing with distilled water, the sections were ground thoroughly with quartz sand at 3° with a mortar and pestle. The homogenates, 7.0 ml, were centrifuged at about $170 \times g$ for 5 minutes. The sediments, which appeared to be mainly cell wall material, were washed with water, 0.01 N NaOH and finally with water again 3 times. The supernatant fractions from the first 2 washings, combined with the original supernatant materials, constituted the "supernatant fraction," and the washed sediment, the "sediment fraction." The proteins were precipitated by adding 2 volumes of 20 % trichloracetic acid and purified by successive washing with 5 % trichloracetic acid, ethanol and ethanol-ether (see 21). During the second wash with 5 % trichloracetic acid the suspension was heated to 90° for 15 minutes. The amounts of ethanol-soluble C^{14} were determined by extracting small samples of the homogenates in hot 80 % (v/v) ethanol. All samples were plated and counted in a Nuclear-Chicago gas flow counter with an efficiency of about 31 %.

Hydrolysis of the C^{14}-Labelled Protein and Paper Chromatography of the C^{14}-Amino Acids. Samples of the proteins labelled with C^{14}-proline were autoclaved with 5 N HCl in sealed glass tubes at 121° for about 24 hours. After drying, the hydrolyzates were extracted with 80 % ethanol. Small samples of the extracts were spotted on to strips of Whatman No. 1 chromatography paper and run for about 24 hours in phenol-H_2O (100: 30 w/w) or acetic acid-*n*-butanol-H_2O (9: 1: 2.9 v/v).

The proline, hydroxyproline, and glutamic acid were located on controls treated with unlabelled amino acids by spraying with ninhydrin. In most cases the radioactivity in the proline and hydroxyproline spots was high enough to allow their location directly with a strip scanning device. The sections of the chromatograms corresponding to proline, hydroxyproline

and glutamic acid were cut off and their radioactivity determined in toluene with a Tri-Carb Liquid Scintillation Counting System at 56 % efficiency.

Determination of Protein Nitrogen. Protein nitrogen was determined with Nessler's reagent, Koch and McMeekin modification, the light absorption being measured with a No. 54 filter on a Klett-Summerson colorimeter. A series containing known quantities of glycine was used as a nitrogen standard and was carried through the same procedure as the unknowns in each experiment.

Measurement of Respiration. The oxygen uptake by 10 artichoke tuber disks was determined with Warburg manometers using 2.80 ml of H_2O. The respiration was measured over a period of 2 to 3 hours with shaking in a constant temperature bath at 25°.

Results

Effect of Auxin on Protein Synthesis. The limited studies which have been made up to the present indicate that auxin does not stimulate total protein synthesis in all cases where it promotes cell enlargement. Since the reported effects on artichoke tuber disks appeared to be the most clear-cut in showing an auxin-induced synthesis of protein (34), these disks were selected for a more thorough study.

Table I shows the time course of the growth of aged artichoke tuber disks in IAA and chloramphenicol. Auxin causes a large increase in the fresh weight of the disks, but little of this takes place in the first 5 hours. The small effect of auxin on growth in the first 5 hours is quite variable and often the auxin-treated tissues did not differ in growth rate from the water controls. A similar delay of about 5 hours before the effect of auxin on growth became apparent was reported by Hanson and Bonner (7) for 2,4-D, while Newcomb (19) observed an even greater delay, up to 48 hours, in tobacco pith. Potato tuber disks, as is well known, also show a lag of close to 48 hours (cf 7, 34). At 24 hours, however, the growth rate of artichoke disks in auxin appears to be maximal and nearly constant. This time was therefore selected to study the effect of auxin and chloramphenicol on the synthesis of protein from C^{14}-amino acids.

Table I. *Time Course of the Effects of IAA and Chloramphenicol on the Growth of Artichoke Tuber Disks*

Incubation		Growth as % increase in fr wt			
		5 hr*	1 day*	2 days*	3 days*
(IAA) mg/liter	(Chlor) mM				
10	0	3.1	29.6	50.9	54.5
10	0.6	2.6	22.8	49.2	58.4
10	1.2	1.2	14.8	25.3	31.8
10	1.8	1.6	7.7	11.2	13.4
10	3.0	1.6	3.8	5.0	6.2
10	6.0	0.8	2.2	3.2	2.8
0	0	1.4	6.0	5.4	6.1

* Following 24-hours' soaking in plain water.

Table II shows that aged artichoke tuber disks treated with auxin for 24 hours incorporate more C^{14}-leucine into protein during a 5-hour exposure than do the controls. The IAA-treated disks also contain correspondingly less ethanol-extractable C^{14}-

Table II. *Effect of IAA (10 mg/liter) on Growth and the Uptake of C^{14}-Amino Acids in Artichoke Tuber Disks*

Growth, % increase in fr wt in 24 hr*	Without IAA 4.7 %	With IAA 22.0 %	% increase
After 5 hr in C^{14}-leucine	Activity in cpm/10 disks		
Supernatant protein**	27,900	32,700	17
Sediment protein**	10,650	16,090	51
80 % ethanol extract	12,710	5,710	— 55
After 5 hr in C^{14}-proline			
Supernatant protein	21,400	25,500	16
Sediment protein	18,370	20,300	10
80 % ethanol extract	14,800	13,400	— 10
Ratio C^{14}-hydroxyproline/ C^{14}-proline*** in:			
Supernatant protein	0.44	0.26	
Sediment protein	3.22	1.78	
C^{14}-proline† from			
Supernatant protein	14,850	20,250	36
Sediment protein	4,350	7,300	68
C^{14}-hydroxyproline† from			
Supernatant protein	6,550	5,250	— 20
Sediment protein	14,020	13,000	— 7

* Following 24-hours' soaking in plain water in all cases.
** Protein fractions obtained as described in Materials and Methods.
*** Separated by descending chromatography in *n*-butanol, acetic acid and H_2O (9:1:2.9).
† Calculated using the ratio of C^{14}-hydroxyproline/C^{14}-proline and the figures for total C^{14}-proline incorporated.

leucine than the controls. The promotion of incorporation into protein is proportionately much greater in the sediment fraction, which contains to a large extent cell wall material, than it is in the supernatant fraction. At the end of the 5-hour exposure to C^{14}-leucine, i.e., 29 hours after the beginning of the auxin treatment, IAA had also increased the total protein in both the supernatant and sediment fractions (table VI); this confirms the earlier observation (34).

As with C^{14}-leucine, IAA also increased the rate of incorporation of C^{14}-proline into protein, and again the increase in the sediment fraction is equalled by the decrease in the alcohol-soluble fraction. However, the effect in the sediment fraction was less than with C^{14}-leucine. Studies on *Lupinus* stems have shown that hydroxyproline is absent, or present in only very small amounts, in the protein of meristematic cells, but is present in large amounts in the enlarged, matured cells (29). Furthermore, large amounts of a hydroxyproline-containing protein have been found to be associated with the cell wall fraction (4, 14). Since this hydroxyproline-containing protein might thus be formed in connection with cell expansion or maturation, it was of interest to examine the effect of auxin on conversion of free C^{14}-proline to protein-bound hydroxyproline. The data in table II indicate that the rate of conversion of free C^{14}-proline to protein-bound C^{14}-hydroxyproline is slightly *less* in tuber disks growing rapidly in IAA than in water controls growing much more slowly, even though the amount of protein-bound C^{14}-proline is greater in the rapidly growing tissue. Similar results were obtained whether the amino acids in the protein hydrolyzates were separated in a phenol-water or butanol-acetic acid-water system, although in the latter the trace of C^{14}-glutamic acid formed ran with the hydroxyproline. As in potato tuber disks (15), much but not all of the hydroxyproline was in the cell wall fraction protein. Some of the hydroxyproline in the supernatant fraction might have been in protein which had been released from the cell walls, or in less readily centrifuged particles. In any case, artichoke tuber disks evidently do not lay down a protein rich in hydroxyproline as a function of growth.

Similar findings were reported for *Avena* coleoptiles in an abstract by Olson et al. (22); there also IAA did not influence the rate of incorporation of C^{14}-proline into protein apparently associated with the cell wall. Thus, in spite of the common occurrence of large amounts of hydroxyproline in cell wall fractions it seems that hydroxyproline-containing proteins are not formed at a greater rate in the cell wall fractions of rapidly growing tissues.

The Inhibition of Growth by Chloramphenicol. The inhibitory effect of chloramphenicol on IAA-induced elongation of etiolated pea stem sections has already been established (fig 1a of ref. 21). To decrease by 50 % the growth increment caused by IAA required about 2.6×10^{-3} M chloramphenicol. Similarly chloramphenicol blocked the growth induced by 2,4-D and by NAA to nearly the same extent in the same sections (table III). Chloramphenicol inhibited elongation in 1 mg per liter IAA to about the same extent as in 10 mg per liter. Since IAA does not limit growth at 10 mg per liter (28 and table III), and the uptake system is not saturated (25), it is very unlikely that chloramphenicol could inhibit growth here through an inhibition of uptake of IAA. Furthermore, chloramphenicol inhibits the endogenous growth of the controls, which is due, at least in part, to endogenous auxin. Thus, although chloramphenicol is certainly able to interfere with the uptake of solutes (10, 23), it evidently does not inhibit auxin action by this means.

Chloramphenicol also inhibited the elongation of *Avena* coleoptile sections, both with and without added IAA (fig 1). As in the case of pea stem sections, the endogenous growth of the controls was not inhibited to so great an extent as the growth induced by added IAA. Chloramphenicol produced a much greater effect on the growth of coleoptile sections at 24 hours than at 5 hours, and the difference between its effects at 5 and 24 hours was even greater than with pea stem sections. Since the coleoptile sections underwent a greater proportion of their growth after the

Table III. *Inhibition of Elongation Induced by IAA, NAA and 2,4-D in Etiolated Pea Stem Sections by Chloramphenicol*

Mean of 2 experiments. The period of incubation was 24 hours.

Chloramphenicol, mM:	0	0.37		3.7	
Auxin	% Elongation	% Elongation	% Inhibition	% Elongation	% Inhibition
None	20	17	15	13	35
IAA 0.01 mg/liter	43	32	26	13	70
IAA 1 mg/liter	56	48	14	26	54
IAA 10 mg/liter	58	50	14	28	52
Chloramphenicol, mM:	**0**	**0.75**		**3.7**	
NAA 1 mg/liter	43	33	23	19	56
NAA 10 mg/liter	62	56	10	32	48
2,4-D 1 mg/liter	42	32	24	15	64
2,4-D 10 mg/liter	54	48	11	28	48

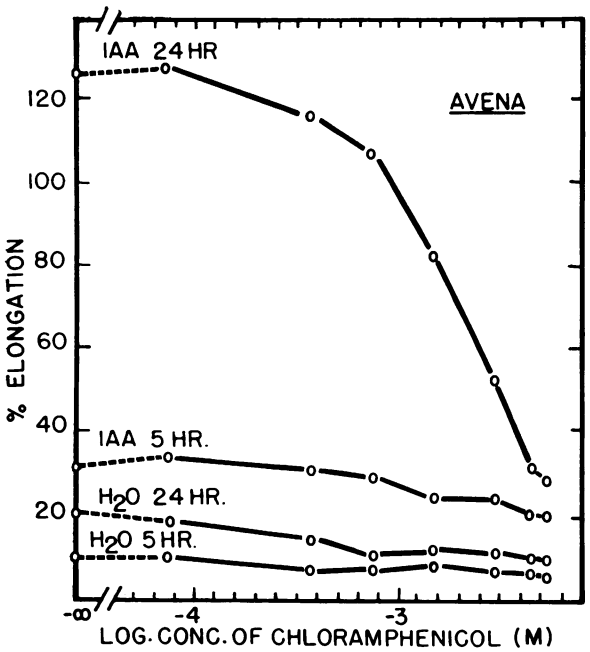

FIG. 1. Inhibition of the elongation of 10-mm oat coleoptile sections by chloramphenicol. IAA 10 mg/liter = 5.8×10^{-5} M.

first 5 hours than pea stem sections did, it appears that chloramphenicol may exert an increasing effect with time. As with pea stem sections, the threshold inhibition of IAA-induced elongation for oat coleoptile sections lay just above 0.10 mM. A 50 % inhibition of the increment of growth induced by exogenous IAA in 24 hours occurred at 1.9 mM chloramphenicol (cf 2.6 mM for pea stem sections). Chloramphenicol also inhibited elongation induced by 2,4-D and NAA in *Avena* coleoptile sections.

As seen already in table I, chloramphenicol has a very striking inhibitory effect on IAA-promoted growth in artichoke tuber disks, i.e., 3.0 mM chloramphenicol completely abolished the effect of added IAA. At 24 hours a 50 % inhibition of the growth induced by exogenous IAA took place at about 1.4 mM. The effect of chloramphenicol on growth induced by NAA in these disks was very similar to that on growth induced by IAA, the 50 % inhibition at 24 hours occurring at 1 mM in 10 mg per liter NAA.

In no case were flaccidity or other signs of general toxicity due to chloramphenicol observed in the tissues studied here. This agrees with the observation (39) that potato disks in 16 mM chloramphenicol were still turgid after 24 hours. It was found, indeed, that treatment of artichoke tuber disks with 6 mM chloramphenicol has no irreparable or permanent effect on their subsequent ability to grow when removed from the drug. Table IV shows that disks treated with 6 mM chloramphenicol and 10 mg per liter IAA for about 24 hours not only recovered their ability to grow after about a day but grew almost as well thereafter as disks treated with water for 24 hours. Recovery of chloramphenicol-treated tissues has been reported earlier by Wilson and Bowen (37), who observed that mitosis in onion roots treated with 3.1 mM chloramphenicol for 12 hours recovered rapidly after tranfer to a medium lacking chloramphenicol.

Table IV. *Recovery of Growth in Artichoke Tuber Disks After Inhibition by Chloramphenicol*

	Growth as % increase in fr wt in:				
(IAA) mg/liter	0	0	10	10	10
(Chlor) mM	0	0	0	6	6
1 day	6.8	6.8	30.0	4.1	2.3
Transfer at the end of the first day to:					
(IAA) mg/liter	0	10	10	10	10
(Chlor) mM	0	0	0	6	0
2 days	6.7	11.4	43.5	4.0	4.8
3 days	7.2	36.3	58.8	4.9	20.8

Inhibition of Protein Synthesis. In the earlier paper chloramphenicol was shown to inhibit the incorporation of C^{14}-leucine into protein in etiolated pea stem sections (fig 1 of ref. 21). Similar concentrations of chloramphenicol inhibited the incorporation of C^{14}-leucine into the protein of oat coleoptile and artichoke tuber sections, to about the same extent as in pea stem sections. For example, in pea stem and oat coleoptile sections exposed to IAA plus C^{14}-leucine for 5 hours, the incorporation of C^{14} into the supernatant protein was reduced to 50 % by 4.2 and 4.3 mM chloramphenicol, respectively. More extensive data for the *Avena* coleoptile are presented in table V. Furthermore, in artichoke tuber disks auxin produced a net increase in total protein as well as accelerating the C^{14}-leucine incorporation, and table VI shows that this increase in protein N caused by IAA was completely blocked in both fractions by 6.0 mM chloramphenicol. At this concentration of chloramphenicol the IAA-induced elongation was also completely inhibited.

Table V. *Effect of Chloramphenicol (Chlor) on the Uptake of C^{14}-Leucine and its Incorporation into Protein in Oat Coleoptile Sections in IAA 10 mg/liter*

The sections were incubated with C^{14}-leucine for 5 hours. Data the mean of 5 experiments.

	Supernatant fraction protein		Ethanol extract
(Chlor) mM	Activity, cpm/ 50 sections	% Inhibition	% Inhibition
0	8,040	0	0
0.075	...	— 2.8	3.8
0.37	7,520	6.5	5.2
1.5	5,910	26	15
3.0	4,750	41	22
5.2	3,560	56	37

Table VI. *Effect of Chloramphenicol (Chlor) on Protein Nitrogen Content and on Incorporation of C^{14}-Leucine into Protein in Artichoke Tuber Disks*

The disks were incubated with C^{14}-leucine for 5 hours starting at 24 hours after the beginning of the treatment with IAA and/or chloramphenicol. The total nitrogen and C^{14} in the protein were determined on aliquots from the same sample. The data are the average of 2 complete experiments.

(IAA) mg/liter	0	10	10
(Chlor) mM	0	0	6
Supernatant fraction protein			
Protein N (mg/10 disks)	0.18	0.22	0.18
Activity (cpm/10 disks)	30,300	41,000	25,700
Sediment fraction protein			
Protein N (mg/10 disks)	0.077	0.089	0.067
Activity (cpm/10 disks)	9,170	14,400	5,160

As is often but not always the case with other solutes (9, 23, 31, 35), the uptake of C^{14}-leucine appears to be inhibited by chloramphenicol to some extent. It had only a small effect on the amount of ethanol-soluble C^{14}-leucine in pea stem sections after a 5-hour incubation (21), but table V shows that in *Avena* coleoptile sections chloramphenicol produced a substantial decrease in the ethanol-extractable C^{14}-leucine (and thus presumably in the leucine uptake). Nevertheless, the incorporation of C^{14}-leucine into protein was inhibited to a markedly greater extent than the C^{14}-leucine uptake. In addition, chloramphenicol clearly decreased the incorporation of C^{14}-leucine into protein even when the sections had been preincubated in a C^{14}-leucine solution for 2 hours, washed and then exposed to chloramphenicol for 4 hours (table VII). In these coleoptile sections chloramphenicol seemed to spare the free C^{14}-leucine in the tissue. The inhibitory effects of chloramphenicol were quantitatively less in such preincubation experiments, probably because considerable amounts of C^{14}-leucine had been bound into protein before the chloramphenicol was added, or before it reached inhibitory levels inside the cells.

Effects of Auxin and Chloramphenicol on Respiration in Artichoke Tuber Disks. At 6 mM, chloramphenicol completely prevented the large increase of O_2 uptake caused by auxin during a 24-hour treatment, with only a small effect on the respiration in the water controls (fig 2). Thus a concentration of chloramphenicol which normally causes inhibition of protein synthesis in plants suppressed the auxin promotion of respiration and produced a similar inhibition of auxin-induced growth. The large increase in respiration caused by auxin in artichoke tuber disks has been ascribed to the synthesis of respiratory enzymes (34) and the fact that chloramphenicol prevents the increase in respiration confirms this.

Since auxin causes an unusually large promotion of respiration and protein synthesis in artichoke tuber disks, the additional protein could be largely respiratory enzymes and thus the connection with the enlargement process may not be direct.

Several reports on the effects of chloramphenicol on respiration in intact plants indicate that it has little or no effect on respiration within the first few hours, although it sometimes becomes inhibitory after prolonged exposures (23, 31, 35). This behavior might be due in part to relatively slow entry of the large and markedly polar molecule. Its relatively slow action even on floating sections was noted above.

Table VII. *Inhibition by Chloramphenicol (Chlor) of the Incorporation of C^{14}-Leucine into Protein of Oat Coleoptile Sections Preincubated in C^{14}-Leucine*

The coleoptile sections were incubated 2 hours in C^{14}-leucine, then 4 hours in IAA (with or without Chlor) without leucine.

(IAA) mg/liter	(Chlor) mM	Activity in cpm/50 sections	
		Supernatant fraction protein	Ethanol extract
10	0	4,850	1,070
10	4.5	3,730	1,400

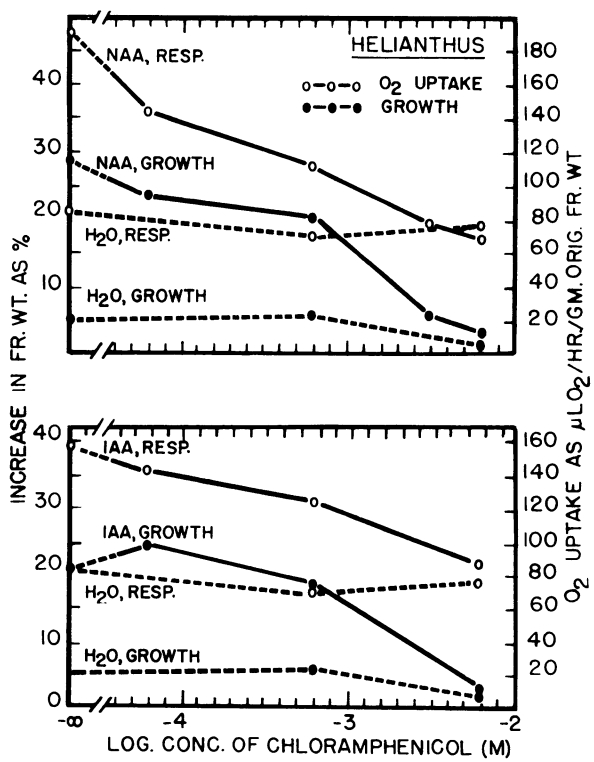

FIG. 2. Effect of chloramphenicol on the growth and respiration induced by IAA (*below*) and NAA (*above*) in artichoke tuber disks. Growth and respiration measured at 24 hours after the start of the treatment with auxin and/or chloramphenicol. Solid lines, auxin; dashed lines, water. Auxins, (IAA) and (NAA), 10 mg/liter.

More probably, however, it is due to the fact that respiration as such is not affected by chloramphenicol, but where an inhibition does occur it is because of the need for protein syntheses (i.e., formation of enzymes) in order to sustain respiration over long periods.

It should be noted that Stoner et al. (30) have recently reported that high concentrations of chloramphenicol interfered with several processes (including O_2 uptake, but not all involving oxidative energy), in isolated plant mitochondria, and also decreased adenosine triphosphatase activity. The effects on isolated mitochondria could be explained through an inhibition of protein synthesis. In any case, it is hardly likely that the mitochondria inside the sections of plant organs used here could have been exposed to such high levels of chloramphenicol, owing to its slow penetration (see the discussion below).

The Relationship between Inhibition of Protein Synthesis and Cell Enlargement. Having established that chloramphenicol functions as an inhibitor both of protein synthesis and of auxin-induced cell enlargement, it remains to compare the 2 effects quantitatively. As was shown earlier for etiolated pea stem sections (21), it is now clear with *Avena* coleoptile sections also that chloramphenicol inhibits the incorporation of C^{14}-leucine into protein, and elongation in presence of IAA, to very nearly the same extent. Figure 3 compares the incorporation of C^{14}-leucine into supernatant protein in 5 hours with the growth in 5 or 24 hours at various concentrations of chloramphenicol. Both these data and those given previously for pea stem sections show a remarkable similarity between the degree of inhibition of IAA-induced cell expansion and protein synthesis in the presence of IAA. At 5 hours, chloramphenicol causes a 50 % inhibition of the coleoptile growth induced by added IAA *at 4.1 mM* and of C^{14}-leucine incorporation into the supernatant protein in presence of IAA *at 4.3 mM*.

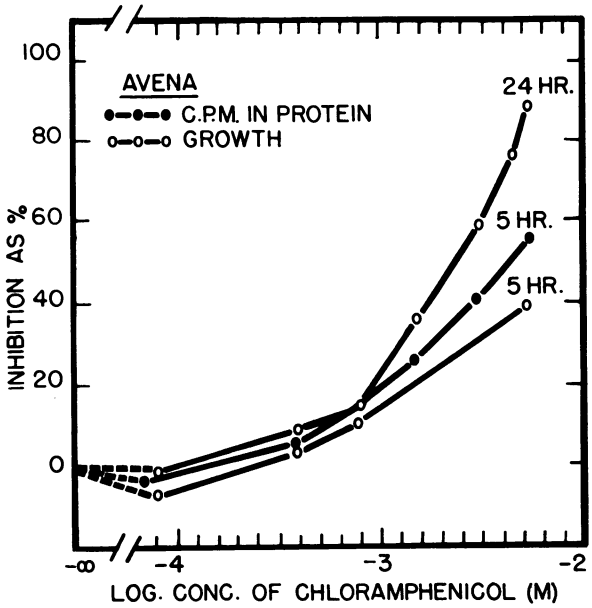

FIG. 3. Comparison of the percentage inhibition of elongation and incorporation of C^{14}-leucine into protein of oat coleoptile sections in the presence of IAA.

Discussion

Chloramphenicol evidently inhibits auxin-induced growth very reproducibly under a variety of conditions. The concentrations of chloramphenicol required to produce a 50 % inhibition of growth induced by added IAA over a 24-hour period are similar for all 3 tissues studied; 1.4, 1.9 and 2.6 mM for artichoke tuber, oat coleoptile and pea stem sections, respectively. Higher concentrations, 4.1 and 5.0 mM, were necessary for 50 % inhibition with oat coleoptile and pea stem sections, respectively, in 5 hours. In the case of artichoke tuber disks, where auxin has very little effect on growth in the first 5 hours, 3.0 mM chloramphenicol is sufficient to maintain complete inhibition of auxin-induced growth for 3 days. Perhaps the greater inhibiting effectiveness on growth in artichoke tuber disks is a result of the relative rates at which auxin and chloramphenicol take effect. If much of the protein synthesis necessary for the induction of cell expansion had taken place before the chloramphenicol reached an inhibitory level inside the tissue, chloramphenicol would be a less effective growth inhibitor.

Since the interpretation of the data on growth inhibition depends on whether or not chloramphenicol inhibits protein synthesis in plants, it may be useful to summarize briefly the evidence for this. First, we must note that chloramphenicol prevents the formation of several enzymes or enzyme systems in whole plants or plant organs (8, 16, 38). Secondly, it specifically inhibits the binding of C^{14}-amino acids into protein in several types of plant material; microsomes (18, 27), protein bodies from developing wheat endosperm (18), and nuclei (11). With microsomal particles from maize kernels, 0.64 and 1.24 mM chloramphenicol produced 27 and 76 % inhibition of C^{14}-leucine incorporation, respectively (27). An exception is offered by nuclei isolated from tobacco cell cultures, however, for 6.2 mM chloramphenicol failed to inhibit the incorporation of C^{14}-lysine into protein in these (5). Perhaps the RNA template for synthesis of basic proteins is stable in these nuclei and thus less affected by chloramphenicol; the nuclei were resistant to ribonuclease also. Thirdly, chloramphenicol inhibited the incorporation of C^{14}-leucine into protein in each of the 3 tissues studied here and it prevented the IAA-induced increase in total protein N of artichoke tuber disks. It can be concluded that chloramphenicol is a powerful inhibitor of protein synthesis in plant tissues. It should be noted too that in the present experiments it apparently also inhibited the uptake of the C^{14}-leucine, though to a lesser extent.

In general, higher concentrations of chloramphenicol are required to inhibit protein synthesis in plants than in bacteria or cell-free bacterial systems. Animal cells and cell-free systems also seem to be relatively resistant to chloramphenicol (6, 36), although concentrations of chloramphenicol as low as 1 μM inhibit polyU-primed C^{14}-phenylalanine incorporation by a ribosomal system from rabbit reticulocytes, providing the chloramphenicol is added before the polyU (36). It may be that plant ribosomes have a lower affinity for chloramphenicol than bacterial ribosomes, but another factor which may contribute to chloramphenicol resistance in plants could be its slower penetration. For example, in *Nitella*, which consists of single large cells, the internal concentration of chloramphenicol is only about 30 % of the external concentration after 2 hours and about 80 % after 6 hours (26). Obviously this penetration problem would be magnified in multicellular systems. Some evidence for relatively slow penetration into the tissues used here was given above.

If we conclude, as seems unavoidable, that some protein synthesis is necessary for auxin-induced cell expansion, the problem is, what type of protein can this be? As has been pointed out, auxin does not necessarily increase the total protein N or rate of protein synthesis in all cases where it promotes cell expansion. Since plant cells may enlarge their vacuoles without any net increase in cytoplasm, it does not appear necessary for the protein content of the cells to increase during expansion. Auxin may cause the synthesis of new proteins without altering the overall rate of protein synthesis either A) if the amount of new protein synthesis is small relative to the total rate of synthesis, or B) if the existing synthesis is redirected, i.e., changes qualitatively. This problem will be considered more fully in a subsequent paper.

The time lag in auxin action, several minutes for oat coleoptile sections (28) and about 5 hours for aged artichoke tuber disks (7), suggests that some metabolic reactions must precede auxin-induced cell expansion. In the case of oat coleoptile sections Ray and Ruesink (28) have demonstrated that the reactions which take place during the lag have a Q_{10} of about 2, as would be expected for chemical reactions. The induction period in oat coleoptile sections is not due to slow uptake of IAA, for the onset of growth is not hastened by increasing the auxin concentration 10-fold.

Several years ago, Masuda (17) showed that a 60-minute pretreatment of oat coleoptile sections with ribonuclease made them unresponsive to IAA within the next 60 minutes. In onion roots ribonuclease produced almost total suppression of protein synthesis in the cytoplasm, probably through destruction of the transfer RNA needed for protein synthesis (12). Masuda's data could be interpreted as showing that auxin can act only when protein is being actively synthesized. Recently, Cleland (3) has reported that oat coleoptile sections pretreated with 1 mM hydroxyproline for as long as 21 hours were still able to respond to IAA; however, the role of hydroxyproline as an inhibitor of protein synthesis is uncertain (32) and it is evidently incorporated to a very small extent, if at all, into the protein of carrot tissue explants (24). Similar studies with some of the antibiotics known to inhibit protein synthesis would be of greater interest. So far it appears that IAA does not act on a protein which has already been synthesized.

Taken together, the failure of IAA to act under conditions where protein synthesis is almost completely inhibited, the striking parallel between the inhibitions of protein synthesis and of auxin-induced growth, and the requirement for an induction period during which metabolic reactions must take place, would support the hypothesis that IAA activates the formation of one or more new enzymes which act on the cell wall to increase its plasticity.

Summary

Chloramphenicol inhibits cell expansion induced by indoleacetic acid and synthetic auxins in a variety of tissues, pea stem, oat coleoptile and artichoke tuber sections. The large promotion of respiration caused by auxin in aged artichoke tuber disks was completely blocked by chloramphenicol, whereas the established respiration of the water controls was not impaired. No signs of general toxicity were observed and artichoke tuber disks treated with chloramphenicol and then washed were able to recover their ability to respond to auxin.

The same concentrations of chloramphenicol which inhibit auxin-induced growth also inhibit protein synthesis in the tissues studied. The percentage inhibition of protein synthesis by a given chloramphenicol concentration was very closely similar to the percentage inhibition of auxin-induced growth in oat coleoptile sections. Continued protein synthesis is therefore considered to be essential to auxin-induced cell enlargement. All the evidence is consistent with the hypothesis that auxin acts by inducing the formation of new proteins or enzymes.

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