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AUXIN-INDUCED GROWTH OF TUBER TISSUE OF JERUSALEM ARTICHOKE

II. THE RELATION TO PROTEIN AND NUCLEIC ACID METABOLISM

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The following results were obtained using tissue slices excised from cold-stored Jerusalem artichoke tuber.

1. Increase in protein content of the tissue was small during the washing (i.e. "aging"), and great in the growth phase, particularly in washed tissue.
2. RNA content of tissue increased during the growth period similarly in non-growing tissue (in water) and actively growing tissue (in 2,4-D plus KIN).
3. Both RNA and DNA increased during the washing, the increase being greater in RNA than in DNA. This RNA increase was enhanced by gibberellic acid.
4. 2-Thiouracil, 8-azaguanine, puromycin, and mitomycin C given at the washing inhibited the subsequent growth. The effect of these inhibitors was not significant when they were given in the growth period.
5. Mitomycin C reduced the basophilia of nuclei and made them swell, as did deoxyribonuclease.
6. The effect of inhibitors of nucleic acid metabolism was reversed to some extent by gibberellic acid and by kinetin.
7. Chloramphenicol inhibited the growth strongly if given in the growing period, but not so strongly if given during the washing.
8. An autoradiographic study using ^{3}H-cytidine suggested that RNA is synthesized in nucleus during the period of washing and is transferred to cytoplasm via nucleolus.

It is conjectured that the RNA synthesized during the aging is responsible for the expansion growth to be caused later by auxin or auxin plus kinetin.

It was shown in the preceding paper (*1*) that the cold-stored tuber tissue of Jerusalem artichoke became prepared for its expansion growth by washing or "aging" of the tissue, the washing with GA solution being especially effective. SETTERFIELD (*2*) and MASUDA and SETTERFIELD (*3*) have suggested that in the tuber tissue the aging process, i.e., the process preparatory

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, kinetin; GA, gibberellic acid; CM, chloramphenicol; TU, 2-thiouracil; AG, 8-azaguanine; PM, puromycin; MC, mytomycin C; RNAase, ribonuclease; DNAase, deoxyribonuclease, RNA, ribonucleic acid; DNA, deoxyribonucleic acid; TCA, trichloroacetic acid.

for the auxin-induced expansion growth, involves an active nucleic acid synthesis. On the other hand, NOODÉN and THIMANN (*4, 5*) have postulated that a specific protein(s) functions in the mechanism of auxin action of inducing the expansion growth. It may then be supposed that GA and auxin play roles somewhere in a chain of reactions from nucleic acid metabolism to protein synthesis.

The present study was designed to elucidate the processes of aging and of tissue expansion in particular connection with protein and nucleic acid metabolism.

MATERIAL AND METHODS

The sources of substances used were: 2,4-D and KIN, Tokyo Kasei Co.; GA, 94% purity, Kyowa Hakko Co., a gift from the company; CM, Sankyo Co.; TU, Nutritional Biochemical Corp.; AG, Tokyo Kasei Co.; PM, American Cyanid Co., a gift from Dr. SAITO, Dept. of Biochemistry of our University; MC, Kyowa Hakko Co., a gift from the company; DNAase and RNAase, Worthington Biochemical Corp.

Preparation of tissue slices of Jerusalem artichoke tuber and growth conditions were as reported previously (*1*). The H_2O-washed (H-W) and GA-washed (G-W) tissues were prepared by immersing tissue slices in distilled water and 5 mg/liter GA solution, respectively, with aeration for 15–20 hr at room temperature (25°). Tissue slices were then put on filter paper held at the surface of growth solutions, i.e. H_2O, 1 mg/liter 2,4-D, 1 mg/liter KIN and 1 mg/liter 2,4-D plus 1 mg/liter KIN. For non-washed (N-W) tissue, excised slices were brought to this condition without washing.

Biochemical determinations were performed as follows:

1. Protein: Tissue was homogenized thoroughly with quartz sand in a cold mortar. TCA was added to supernatant to give a final concentration of 5%. Precipitated protein was measured after dissolved in a small amount of 1 N NaOH solution (*6*).

2. Nucleic acids: Tissue was homogenized in the cold, and RNA and DNA were extracted by the SCHMIDT-THANNHAUSER method (*7*). RNA- and DNA-phosphorus were determined indirectly from OD at 260–290 mμ (Tables II and III) or directly after digestion with perchloric acid (*8*) (Table I).

3. Autoradiography: N-W tissue was fed with 5 μc/ml ^{3}H-cytidine (specific activity, 1.3 c/mmole) for 2.5 hr at 25°, washed and transferred to 20 mg/liter cold cytidine solutions containing and not containing 100 μg/ml RNAase. A part of the tissue slices incubated in the cold cytidine solution without RNAase were transferred after 20 hr to fresh cold cytidine solutions containing and not containing RNAase. Experimental procedure for autoradiography was essentially the same as reported by SETTERFIELD (*2*). The data are presented as mean grain counts over 10 nuclei and 10 cytoplasmic regions in each of three different tissue slices.

RESULTS

Protein metabolism in connection with expansion growth

NOODÉN and THIMANN (*4, 5*) maintain that specific protein is required for the auxin action in Jerusalem artichoke. Firstly in the present study, changes in the amount of protein were measured for N-W, H-W and G-W tissues. Results are illustrated in Fig. 1.

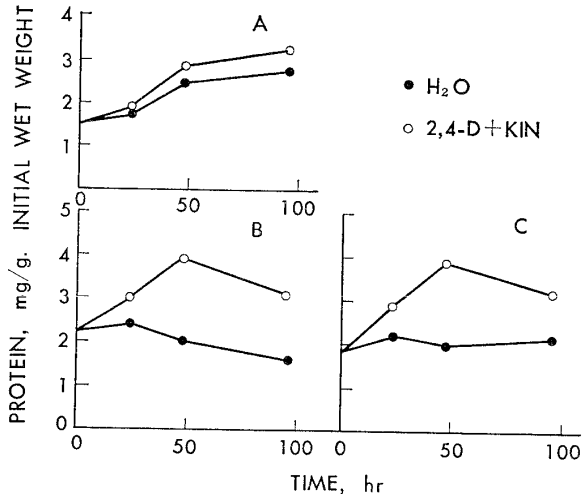

Fig. 1. Time course of changes in amount of TCA-precipitable protein of N-W (A), H-W (B), and G-W (C) tissues.

With N-W tissue, TCA precipitable protein increased with expansion, and the increase was slightly but significantly enhanced by 2,4-D plus KIN (Fig. 1A). When tissue slices were washed, either in the presence (Fig. 1C) or absence (Fig. 1B) of GA, protein increased during the washing (for 17 hr), just corresponding to the protein increase in the N-W tissue growing in water (Fig. 1A). In H-W and G-W tissues the amount of protein did not change or even decreased slightly in the absence of the growth regulators, but increased conspicuously in the presence of 2,4-D plus KIN for the first 50 hr, then began to decrease somewhat. The above results with the washed tissues are very similar to those reported by THIMANN and LOOS (*9*). It is to be studied whether the increase of protein is only a result of growth or it means the synthesis of a specific enzyme(s) required for the auxin action to promote expansion growth.

N-W slices were incubated in 2,4-D and 2,4-D plus KIN solutions to which CM was added at various concentrations. The expansion growth determined after 96 hr is plotted in Fig. 2. Ten mg/liter CM produced 50% inhibition of expansion growth in the presence of 2,4-D alone. Protein synthesis seems to be necessary for expansion growth. KIN seemed to alleviate the inhibitory effect of CM, in favor of MOTHES (*10*) who has

reported the KIN-CM competition in tobacco leaf. CM is considered to block protein synthesis by attaching to ribosomes (e.g. *11*). Then, KIN might compete with CM at the ribosomal level.

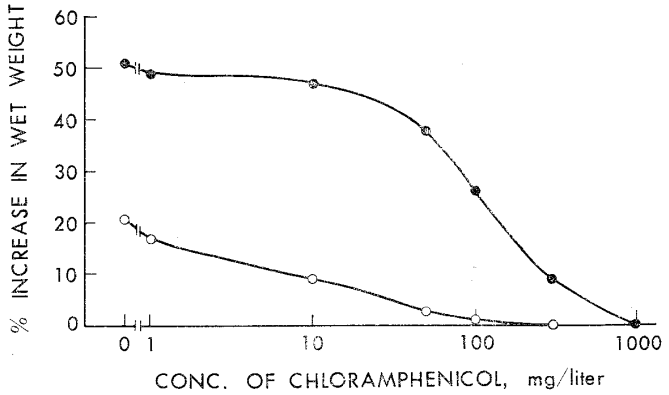

Fig. 2. Effect of CM at various concentrations on the expansion growth of N-W tissue in the presence of 2,4-D (○) and 2,4-D plus KIN (●). Incubation period, 96 hr.

In order to see how the inhibition of protein synthesis during the aging period affects the growth to follow, tissue slices were washed with 10^{-3} M (323 mg/liter) CM for 17 hr, rinsed in distilled water 3 times and transferred to 2,4-D and 2,4-D plus KIN solutions. These slices started to grow later and grew more slowly than H-W slices (Figs. 3B and A). The presence

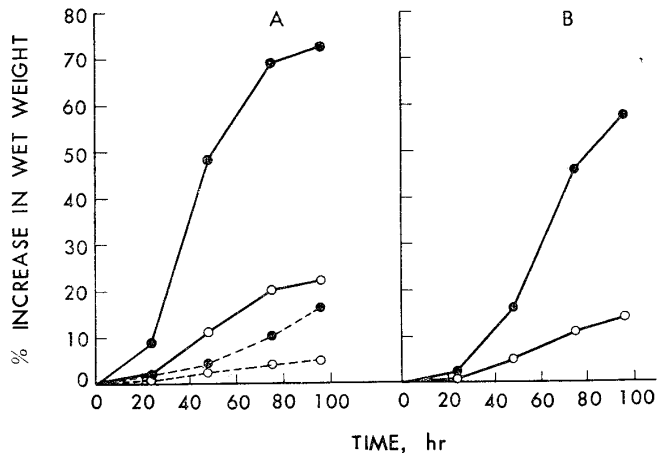

Fig. 3. Effect on the expansion growth of 10^{-3} M CM given in the washing period (B) and in the growth period. (A). A: Time course of expansion growth of H–W tissue caused by 2,4-D (○) and 2,4-D plus KIN (●) in the presence (------) and absence (——) of CM. B: Time course of expansion growth of CM-wash tissue caused by (2,4-D) and 2,4-D plus KIN (●).

of the same concentration of CM in the growth medium inhibited the expansion seriously (Fig. 3A).

Experimental results so far described are consistent with the notion that the protein formation is necessary for the auxin-induced expansion growth to take place (*4, 5, 12*). Shorter lag period and higher growth rate in H-W and G-W tissues than in N-W tissue (1, Figs. 1, 2, and 3) may probably be ascribed to the enzyme synthesis occurring during the washing and in the subsequent lag period.

Nucleic acid metabolism in connection with expansion growth

In connection with the protein metabolism, nucleic acid metabolism in the aging period and in the growth period should be investigated. H-W and G-W tissues were grown on H_2O and 2,4-D plus KIN for 96 hr, and RNA was extracted. The results presented in Table I show that, although the

TABLE I

Effect of 2,4-D plus KIN applied for 96 hr on the RNA content of H-W and G-W tissues

RNA fraction was digested in perchloric acid to be measured for phosphorus content. Mean values of 4 parallel experiments, with standard errors, are indicated.

Tissue	RNA-P, μg/g initial wet tissue	
	H_2O	2,4-D plus KIN
H-W	329±57.8	299±24.7
G-W	346±44.6	357±20.5
Initial	16.6±3.08	

TABLE II

RNA-P, DNA-P and protein contents in differently aged tissues, N-W, H-W and G-W

Nucleic acid-P was calculated upon OD at 260–290 mμ assayed for RNA and DNA fractions. The contents per g initial wet tissue are indicated. Mean values of 4 parallel experiments, with standard errors, are indicated.

Expt. No.	Tissue	RNA-P, μg/g	DNA-P, μg/g	RNA/DNA	Protein, mg/g
1	N-W	286± 8.8	71.4±1.22	4.0	1.3
	H-W	332±10.5	75.6±1.18	4.5	2.2
	G-W	399± 9.2	79.8±0.97	5.0	2.0
2	N-W	202±11.8	58.8±3.02	3.5	1.5
	H-W	298±21.1	75.6±7.56	3.8	2.3
	G-W	290±24.4	71.4±5.04	4.1	1.8
3	N-W	298± 7.6	79.8±5.04	3.7	1.5
	H-W	391±23.5	92.4±8.40	4.2	2.2
	G-W	424±11.8	100.8±3.78	4.2	1.8

RNA level was roughly doubled in 96 hr of growth, it was not affected significantly by the presence of 2,4-D plus KIN.

DNA, RNA and protein contents of washed tissues were studied (Table II). During the aging, RNA increased appreciably whereas DNA increased only slightly. The increase in RNA was more conspicuous in G-W than H-W (see also the ratio RNA/DNA in the table). Protein increased during the aging, and the increase was greater in H-W than in G-W tissue.

It thus seems that, during the aging, active RNA metabolism takes place, which is promoted by GA. At least a part of this newly synthesized RNA might be functional in the auxin action to follow (*13, 14*). To test the hypothesis, effects of several inhibitors of RNA metabolism on the aging and the growth were observed.

Fig. 4 illustrates the effect of TU, given together with 2,4-D and 2,4-D plus KIN, on the growth of N-W tissue. The concentration of TU to give half the maximum inhibition was higher when KIN was present, just as in the case of CM-inhibition (cf. Fig. 2). It is reported also in tobacco leaf that the inhibitory effect of TU is reversed by KIN (*10*).

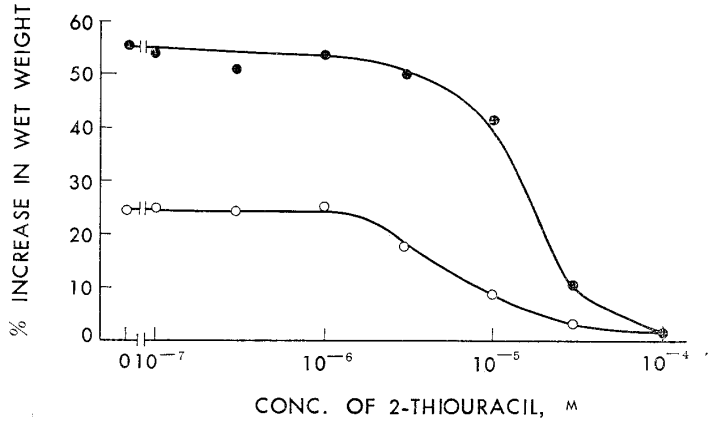

Fig. 4. Effect of TU at various concentrations on the expansion growth of N-W tissue in the presence of 2,4-D (○) and 2,4-D plus KIN (●). Incubation period, 96 hr.

The growth of H-W and G-W tissues was inhibited only slightly by 3×10^{-5} M TU given in the growth phase (Figs. 5A and C), while N-W tissue was inhibited considerably by the same treatment (Fig. 4). When 3×10^{-5} M TU was given during the aging, the growth began to proceed late (Fig. 5B). This effect of TU was somewhat alleviated when GA was given together with TU (Fig. 5D). It is noticed that, if given at the washing or given to N-W tissue, either GA or TU is effective, the effect being mutually opposite. This is in accordance with the assumption that the two agents affect the RNA metabolism which is prerequisite for the growth.

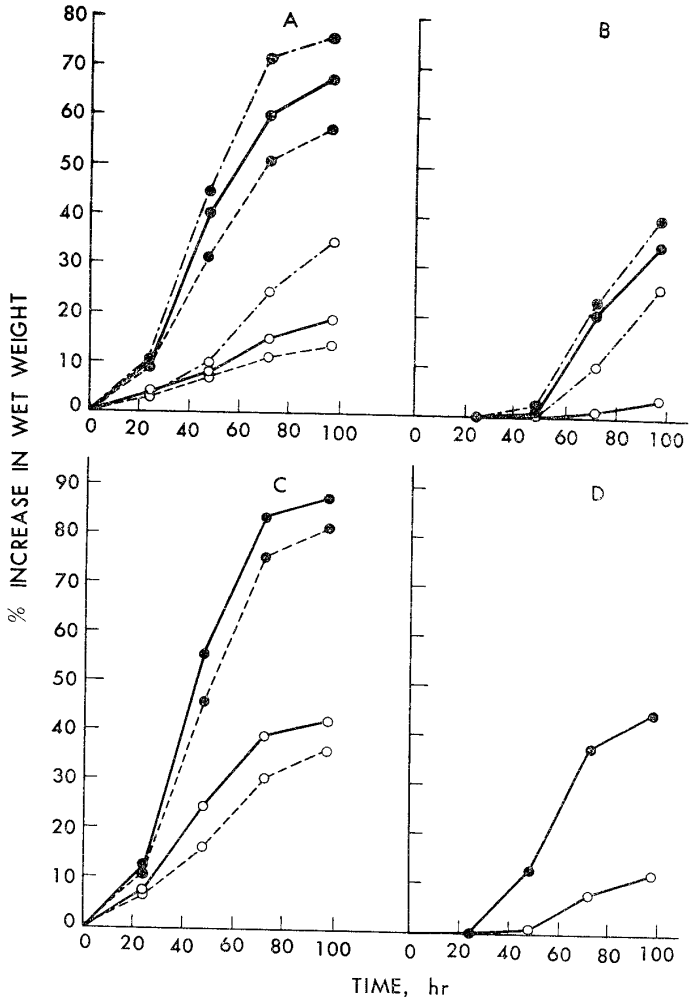

Fig. 5. Effect on the expansion growth of 3×10^{-5} M TU given in the washing period (B, D) and in the growth period A, C). A: Time course of expansion growth of H–W tissue caused by 2,4-D (○) and 2,4-D plus KIN (●) in the presence of TU (------) and 5 mg/liter GA (—·—) and in their absence (——). B: Time course of expansion growth of TU-wash tissue after transference to 2,4-D (○) and 2,4-D plus KIN (●) containing (—·—) and not containing (——) GA. C: Time course of expansion growth of G–W tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing (------) and not containing (——) TU. D: Time course of expansion growth of the tissue washed by GA plus TU after transference to 2,4-D (○) and 2,4-D plus KIN (●).

Fig. 6 shows the effect of various concentrations of AG given to N-W slices in the growing phase. The curves suggest that the inhibitory action of AG is not reversed by KIN. The growth of H-W tissue was not much inhibited by 10^{-5} M AG (Fig. 7A), which inhibited N-W tissue considerably. And AG was effective during the aging period (Fig. 7B). Thus, the case

with AG is the same as with TU. A similar effect has also been reported for 5-fluorouracil (*2*).

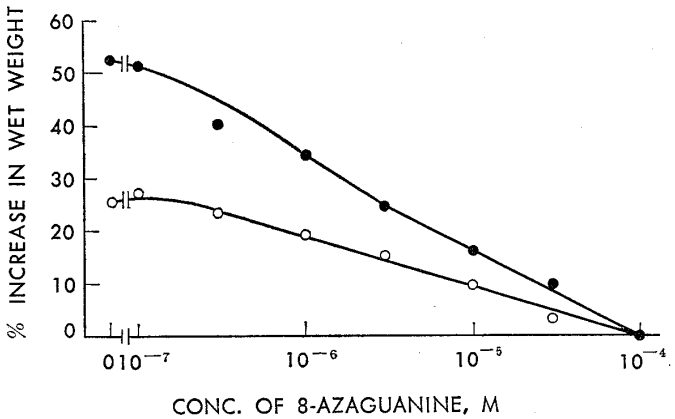

Fig. 6. Effect of AG at various concentrations on the expansion growth of N-W tissue in the presence of 2,4-D (○) and 2,4-D plus KIN (●). Incubation period, 96 hr.

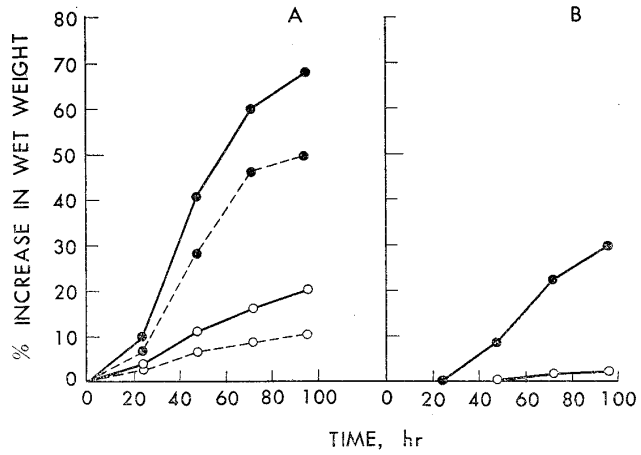

Fig. 7. Effect on the expansion growth of 10^{-5} M AG given in the washing period (B) and in the growth period (A). A: Time course of expansion growth of H–W tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing (------) and not containing (——) AG. B: Time course of expansion growth of AG–wash tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●).

PM, which is known to inhibit the synthesis of ribosomal precursor (cf. *15, 16*), showed essentially the same effect as the base analogs described above (Figs. 8A and B). When KIN was added in combination with PM during the aging treatment, the growth inhibition by PM was reversed a little (cf. Fig. 9B with Fig. 8B). Adenine did not reverse the effect of PM (Figs. 9A and B).

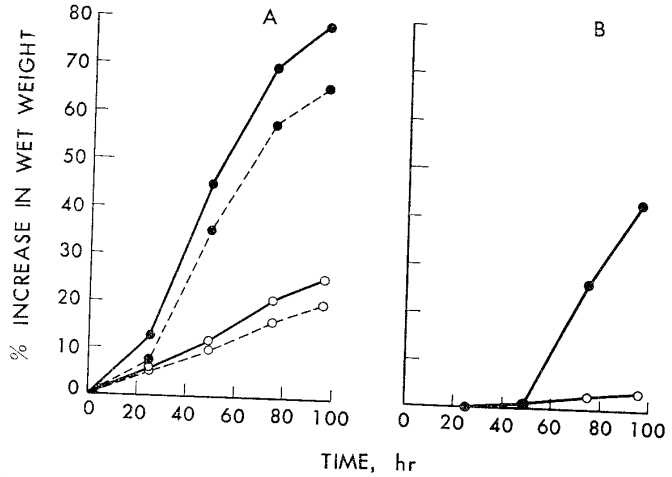

Fig. 8. Effect on the expansion growth of 10^{-4} M PM given in the washing period (B) and in the growth period (A). A: Time course of expansion growth of H–W tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing (------) and not containing (——) PM. B: Time course of expansion growth of PM-wash tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●).

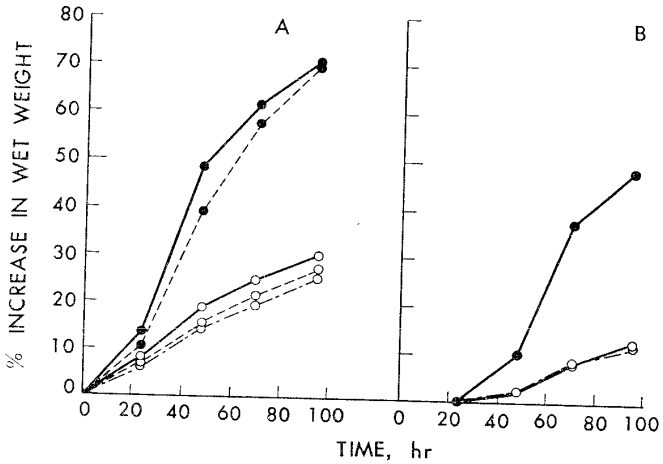

Fig. 9. Effect of KIN on the inhibitory action of PM. A: Time course of expansion growth of KIN-wash tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing 10^{-14} M PM (------), 1 mg/liter adenine (—·—) and neither of them (——). B: Time course of expansion growth of the tissue previously washed with PM plus KIN, after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing (—·—) and not containing (——) adenine.

The experimental results so far described are consistent with the assumption that the process preparatory to auxin-induced expansion growth involves RNA and protein metabolism.

If RNA synthesis is the prerequisite for the growth to occur, impairment occurring in DNA during the aging period will be expected to reflect on diminished growth. Hence the effect of MC, which was reported to degrade or digest DNA (e.g. *17–19*), was examined.

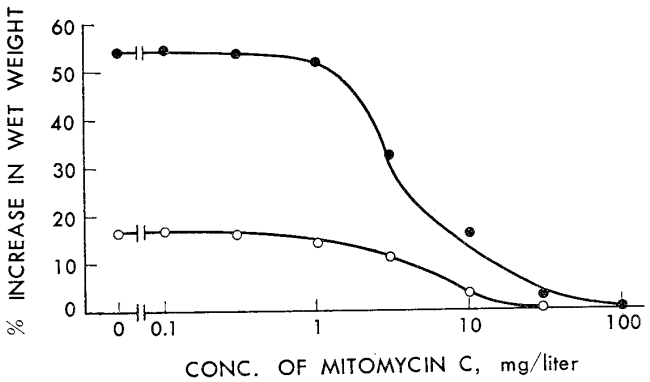

Fig. 10. Effect of MC at various concentrations on expansion growth of N-W tissue in the presence of 2,4-D (○) and 2,4-D plus KIN (●). Incubation period, 96 hr.

With 5×10^{-6} M MC, the growth of H-W and G-W tissues was not inhibited (Figs. 11A and C), while the corresponding concentration (5 mg/liter) inhibited N-W tissue considerably (Fig. 10). Washing with MC made the growth difficult (Fig. 11B). Thus the effect of MC was quite similar to TU, AG and PM. GA reversed the effect of MC (Figs. 11B and C).

Table III shows the results of determination of RNA, DNA and protein of MC-wash tissue, as well as of N-W and H-W tissues. The washing with 5×10^{-6} M MC inhibited the net synthesis of RNA and protein during the aging period and decreased the content of DNA.

Microscopic observation was performed to see if MC exerted a degradative action on the nucleus of artichoke tuber cell just as has been reported for mouse cell (*19*). A thin hand section of tuber tissue was stained with Giemsa solution for 10 min at pH 6.8, washed and mounted in water. Whereas nuclei of H-W tissue cells were stained distinctly (Fig. 12A), those of MC-wash tissue cells were swollen and only poorly stained except nucleolus (Fig. 12B). The nuclear membrane appeared to have disintegrated more or less. KERSTEN (*18*) ascribed the inhibiting effect of MC on bacterial growth to the activation of DNAase. Fig. 12D illustrates a cell nucleus of tuber tissue treated with DNAase, instead of MC, in the presence of 0.003 M $MgCl_2$. Basophilia and the size of nucleus, as well as appearance of disintegrated nuclear membrane, resembled those of the nuclei of MC-wash cells. Nuclei of MC-wash tissue were observed after 50 hr in 2,4-D plus KIN, namely, when the tissue had recoverd the growth activity (cf. Fig. 11B). As seen in Fig. 12C, basophilia and integrity of nuclei had also been restored to the extent that observed in H-W cells (Fig. 12A).

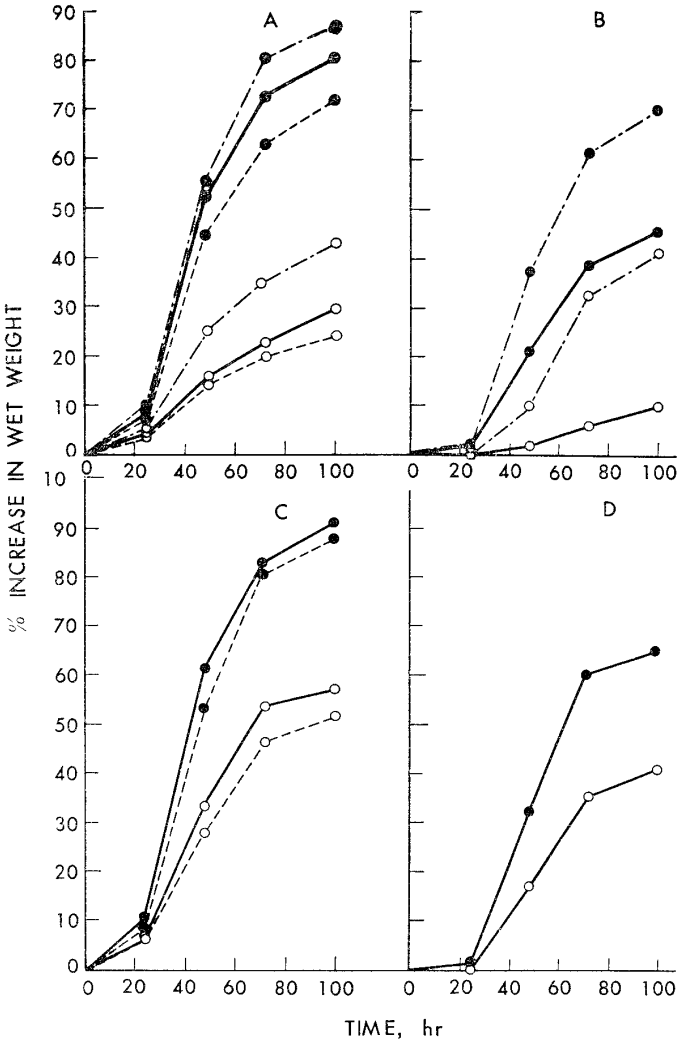

Fig. 11. Effect on the expansion growth of 5×10^{-6} M MC given in the washing period (B, D) and in the growth period (A, C). A: Time course of expansion growth of H-W tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing MC (------), GA (—·—) and neither of them (——). B: Time course of expansion growth of MC-wash tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing (—·—) and not containing (——) GA. C: Time course of expansion growth of G-W tissue after transfer to 2,4-D (○) and 2,4-D plus KIN (●) containing (------) and not containing (——) MC. D: Time course of expansion growth of tissue, previously washed with GA plus MC, after transfer to 2,4-D (○) and 2,4-D plus KIN (●).

Autoradiographic study

An autoradiographic study was performed to gain an insight into the RNA metabolism during and after the aging. Fig. 13 illustrates the results.

TABLE III

Effect of washing with 5×10^{-6} M MC on RNA-P, DNA-P and protein contents of tissue

Nucleic acids in μg per g initial wet tissue were calculated upon OD at 260–290 mμ determined after extraction. Mean values of 4 parallel experiments, with standard errors, are indicated.

Tissue	RNA-P. μg/g	DNA-P, μg/g	RNA/ DNA	Protein, mg/g
Before washing	236±13.6	94.2±1.7	2.5	1.4
After H-W	362±13.5	88.0±1.4	4.1	2.3
After MC-washing	276±27.8	74.4±3.7	3.7	1.8

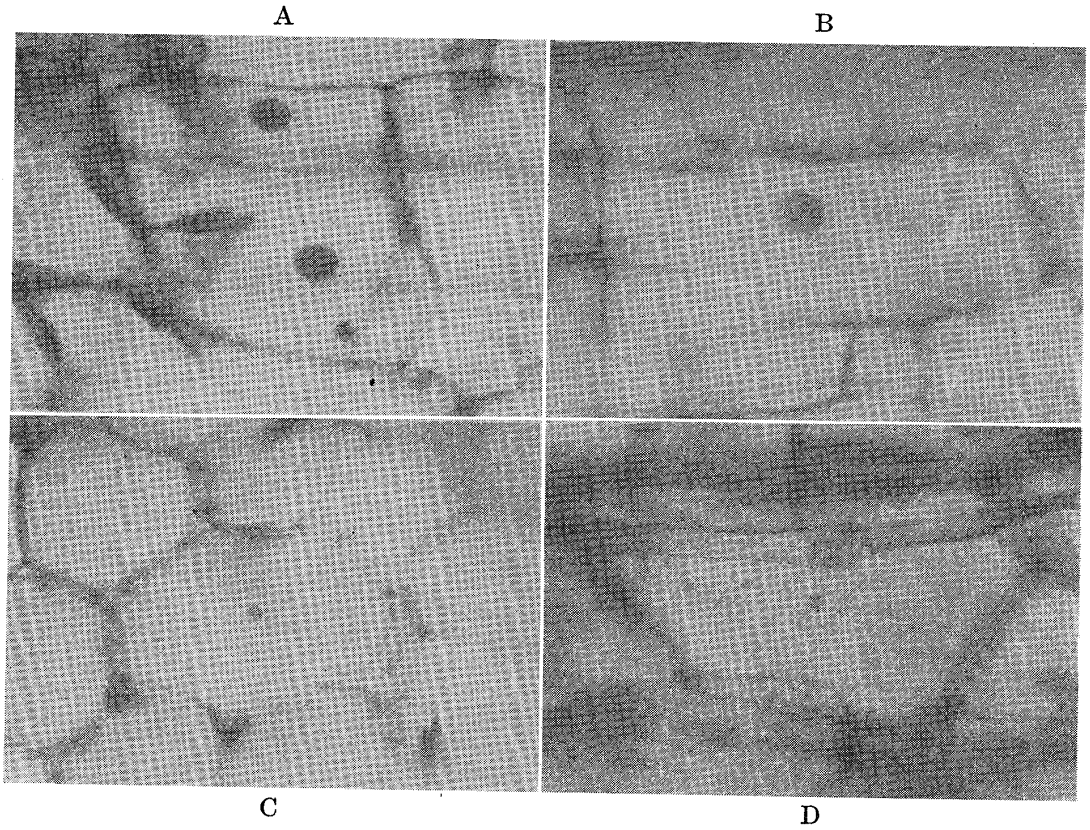

Fig. 12. Effect of washing with 5×10^{-6} M MC and DNAase on the cell nucleus. Staining with Giemsa solution at pH 6.8. A: Cells of H-W tissue; B: cells of MC-wash tissue; C: cells of MC-wash tissue grown on 2,4-D plus KIN for 50 hr; D: cells treated with DNAase (50 μg/ml in the presence of 0.003 M $MgCl_2$).

Incubation with RNAase proved that tritium had been incorporated exclusively into RNA. Cytidine incorporation was rapid and the heaviest in nucleolus, moderate in the chromatin region and very light in cytoplasm (Fig. 13A). The label over nucleolus and chromatin region rapidly decreased with a concomitant gradual increase in cytoplasm when the slices were incubated, namely aged, in cold cytidine. Since a very short-term

labelling revealed silver grains to occur predominantly over the chromatin region (*2*), it is suggested that RNA is synthesized in chromatin and translocated to nucleolus and then to cytoplasm, as reported by others (e.g. *2, 20*). After 20 hr of aging in cold cytidine, no appreciable label was found over nucleolus and the chromatin region, and the label remaining over cytoplasm was not removed any more at least for 8 hr (Fig. 13B). The label was mostly removed when the tissue was treated with RNAase soon after the incorporation (Fig. 13A), but was removed only partly when the enzyme was given after the aging period (Fig. 13B).

The results of experiments using inhibitors of nucleic acid metabolism and autoradiography suggest that the DNA-directed RNA synthesis and the distribution of the synthesized RNA in the cytoplasm are involved in

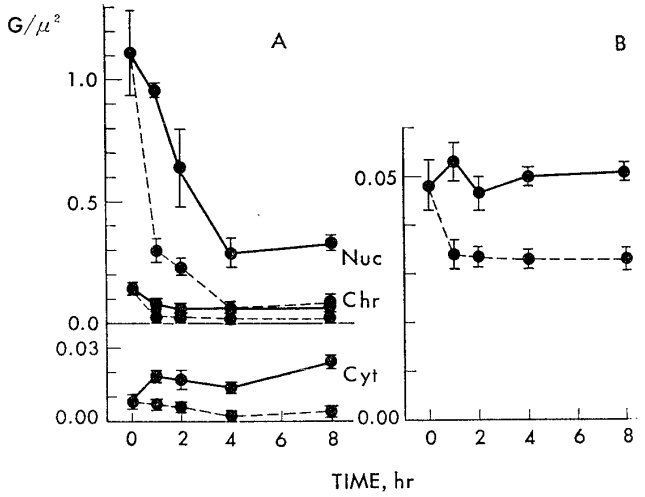

Fig. 13. Time course of changes in the number of silver grains per μ^2 in autoradiograph of the tissue labelled for 2.5 hr with 5 μc/ml ^{3}H-cytidine and, then, chased with 20 mg/liter cold cytidine. Means of 10 nuclei and 10 cytoplasmic regions in each of 3 different tissue slices, with standard errors, are shown. A: Changes in silver grains over nucleolus (Nuc), chromatin region (Chr) and cytoplasm (Cyt) of N-W tissue chased in the presence (------) and absence (——) of 100 μg/ml RNAase. B: Changes in silver grains over cytoplasm of H-W tissue treated with cold cytidine solution containing (——) and not containing (——) RNAase after chasing for 20 hr.

the aging process which prepares for a rapid expansion growth to be caused by 2,4-D and 2,4-D plus KIN.

DISCUSSION

A preparatory process (i.e. previous washing) is needed for the tuber tissue of Jerusalem artichoke to become capable of auxin-induced expansion

growth. The biochemical and biophysical analyses of this preparatory stage may offer a clue to the primary action of auxin.

It is shown in the present study that nucleic acid metabolism is essential for the preparatory process, but not for the expansion growth itself. Net RNA synthesis occurred during the phase of expansion growth, although it was not influenced by auxin plus kinetin (Table I) which stimulated the growth.

In oat coleoptile, 20–30 mm in length, which is very sensitive to auxin but not to gibberellin and is incapable of cell division, the auxin-induced elongation is not inhibited by TU and AG (*8*). In this case, RNA may be synthesized and the cells are already prepared for the action of auxin before the main elongation period. The aged tissue (H-W or G-W) of Jerusalem artichoke may correspond to the oat coleoptile of 20–30 mm length, and N-W tissue of the former to younger coleoptile.

The idea that the RNA synthesis is prerequisite for the expansion growth is supported also by experiments using other materials. Some RNA base analogs were shown to inhibit the growth of embryonic tissues while they had no effect on grown ones (cf. *21*). WOODSTOCK and SKOOG (*22*) found that the capacity of expansion growth of corn roots was directly correlated with the amount of RNA synthesized in the meristematic apex previous to the rapid expansion. HOLMES et al. (*23*) showed that the RNA content per cell reached a maximum in an early elongation stage of broad bean roots.

Synthesis of RNA may be dependent on DNA template. Auxin-induced expansion of artichoke tuber tissue was inhibited by base analogs and PM, just as by actinomycin D (*4*, SETTERFIELD's personal letter). According to SETTERFIELD (*2*), ^{3}H-5-fluorouracil is first incorporated into nuclei of cells of N-W tuber tissue and the label gradually moves into cytoplasm, and as a result the auxin-induced expansion growth is inhibited. All these facts suggest a possibility that synthesis of RNA, perhaps of the messenger type, on the DNA template plays an important role in the expansion growth to be caused by auxin.

KEY and INGLE (*24*) have reported that, in soybean hypocotyl, 5-fluorouracil inhibits the synthesis of ribosomal and soluble RNA, but not the growth and the synthesis of DNA-like RNA. The latter two are inhibited by actinomycin D. The RNA which they postulate as essential for the auxin-induced growth may perhaps correspond to the functional RNA reported in our previous papers (*13, 14*). Some other experiments (e.g. *25*) also suggest that the nuclear RNA, which is not easily extractable and is perhaps DNA-dependent, is important in the auxin action. When YAMAKI (*26, 27*) fed pea homogenate with radioactive IAA for 5 min, the label was recovered exclusively in a particulate fraction, probably ribosomal. And this was not the case when the homogenate was treated with RNAase prior to the IAA feeding. This may suggest that the primary site of auxin action is RNA (or ribosome). Several in vivo (e.g. *3, 28, 29*) and in vitro studies (*30–32*) seem to support the possibility.

In contrast to these observations, some workers found relatively large increases of RNA during (*33, 34*) and after (*35*) the rapid growth. The disagreements may possibly come from differences in material, conditions, and the method of determination.

The role played by the previously synthesized RNA in the expansion growth is not yet known. NOODÉN and THIMANN (*4, 5*) maintain that the auxin-induced growth requires RNA-dependent synthesis of a certain enzyme(s) which occurs during the lag period of the growth.

A considerable number of investigations have been done on the effect of plant hormones on enzyme activities (cf. *36–38*). VARNER and CHANDRA (*39*) have shown that gibberellin controls the synthesis of α-amylase in aleurone cells of barley endosperm by stimulating the production of messenger RNA. Hormonal control of enzyme seems to occur primarily at the nucleic acid level in this case. In the tuber tissue of Jerusalem artichoke, GA promotes the aging process probably by affecting the DNA-dependent RNA metabolism, since GA given in the aging period increases RNA as well as the subsequent expansion growth in the presence of auxin. The hypothesis is supported also by the finding that the RNA fraction recovered in the non-aqueous layer of the phenol-treated extract from G-W tuber tissue has the same effect as GA to make N-W tissue responsive to auxin (*13, 14*).

CM, an inhibitor of protein synthesis, inhibited expansion growth. As for the reported fact that the increase in protein content is not enhanced by auxin in oat coleoptile (*4, 40, 41*), NOODÉN and THIMANN (*5*) suppose that the synthesis of very minor protein is necessary for auxin-induced cell expansion. WRIGHT (*42, 43*) has found in wheat coleoptile that the change with age in the responsiveness to auxin, gibberellin and kinetin is accompanied by the changes in cellular antigen, assumedly enzyme proteins.

It has become highly probable that the DNA-RNA-protein story is involved in the expansion growth of plant tissues. The present paper particularly puts stress on the synthesis of the RNA which is essential for the auxin action on cell expansion (*8, 28, 29*).

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