

# CHANGES IN MICROSOMAL ELECTRON TRANSPORT OF PLANT STORAGE TISSUES INDUCED BY SLICING AND AGING

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## Abstract

Slicing turnip, swede, and beet storage tissues induced 20–100% loss of microsomal NADH dehydrogenase activities within 10 min. Subsequent washing of the slices resulted in partial recovery of some activities particularly NADH-cytochrome *c* reductase which reached a maximum after 24 hr aging then again declined. Slicing also induced a 20% decrease in microsomal protein but this loss was recovered after 5–10 hr aging. These induced changes correlated with reported changes in the ultra-structure of the endoplasmic reticulum.

The induced increases but not initial losses in activities in turnip disks were completely inhibited by cycloheximide and partially by 6-methylpurine. Delayed addition of cycloheximide showed that, although there were net increases in all activities after 8 hr of aging turnip disks, loss of NADH-cytochrome *c* but not NADH-ferricyanide or NADH-2,6-dichlorophenolindophenol reductases was still occurring. However, there was no loss of activities by 12 hr of aging.

Indoleacetic acid ( $6 \times 10^{-6}$ M) induced a specific loss of NADH-cytochrome *c* reductase similar to that induced by slicing. Higher concentrations resulted in losses of all activities and a decreased microsomal yield.

Incubation of the isolated turnip microsomes with the soluble supernatant fraction resulted in losses of NADH dehydrogenase activities. In turnip disks aged for 12 hr the microsomes became partially insensitive but the soluble fraction more inhibitory.

It was concluded that slicing probably induces a release of hydrolytic enzymes from the large central vacuole which act directly on the microsomal membranes. With aging, the membranes are first resynthesized (and the hydrolytic enzymes inactivated) then again degraded.

## I. INTRODUCTION

It is well established that slicing of plant storage tissue induces an immediate increase in respiration, and the ability to accumulate ions develops in these slices with aging (Laties 1967). These induced changes have not been satisfactorily elucidated and cannot be explained by the changes detected in mitochondria isolated from fresh and aged tissue (Hackett *et al.* 1960; Van Steveninck and Jackman 1967). Although cytochrome *b<sub>7</sub>* appears to increase in mitochondria in aging tissue slices, Kahl (personal communication) concluded that electron transfer to oxygen proceeded through the normal, tightly coupled respiratory chain. It has also been shown that the mitochondria isolated from both fresh and aged tissue accumulated ions (Goh and Wiskich 1967).

Hence, induced physiological changes may result from changes in systems other than the mitochondria. A number of changes in the microsomal fraction and the

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endoplasmic reticulum in aging plant storage tissues have been reported. Twofold increases in protein and four- to sevenfold increases in NADH dehydrogenases were detected in the microsome fraction isolated from potato slices aged for 24 hr (Hackett *et al.* 1960; Ben Abdelkader 1969). A sharp increase in newly synthesized phospholipids in the microsome fraction within the first 10 hr of washing potato slices (Tang and Castelfranco 1968) was interpreted as biosynthesis of endoplasmic reticulum.

Changes in the endoplasmic reticulum of beetroot (Jackman and Van Steveninck 1967), carrot, swede (Van Steveninck 1970), and jerusalem artichoke (Fowke and Setterfield 1968) with aging have also been observed, although there did not appear to be a common trend. The endoplasmic reticulum of beetroot formed vesicles 2 hr after slicing, and of swede after about 40 hr. However, with further washing there was a subsequent increase in the endoplasmic reticulum of beetroot and jerusalem artichoke.

This paper describes the changes in the microsomal NADH electron transport system induced by slicing and aging turnip storage tissue. Results are considered in the light of reported changes in the tissue slices, the endoplasmic reticulum, and the microsome fraction.

## II. METHODS

### (a) Preparation of Disks

Disks (1 mm thick) were cut from cylinders (1 cm diameter) of root storage tissue of commercially obtained turnip (*Brassica rapa* L.), swede (*Brassica napobrassica* Mill.), and beetroot (*Beta vulgaris* L.). The disks were well rinsed and aged in  $10^{-4}\text{M}$   $\text{CaSO}_4$ , which was frequently changed and aerated with a stream of filtered air. Disks were rinsed three times in distilled water to remove the  $\text{CaSO}_4$  before homogenizing. When preparing microsomes from fresh disks the period between cutting and homogenizing was 10–15 min.

Respiratory rates of disks were measured at 25°C using standard manometric techniques with air as the gas phase (Umbreit, Burris, and Stauffer 1964).

### (b) Microsome Preparation and Assay

Microsomes were prepared from both whole tissue and disks, and the enzyme activities, cytochrome  $b_3$ , and protein assayed as described by Rungie and Wiskich (1972). Microsomes were usually isolated from 100 g of tissue. In a few experiments where 25 g of tissue was used, homogenizing was by mortar and pestle rather than juice extractor.

### (c) Chemicals

Cytochrome  $c$  (type III from horse heart), NADH, cycloheximide, and 6-methylpurine were obtained from Sigma Chemical Company, St. Louis, U.S.A. Indoleacetic acid was obtained from British Drug Houses, Poole, U.K.

## III. RESULTS

### (a) In vivo Changes in Microsomal Reductases Induced by Tissue Slicing

Microsomal NADH dehydrogenase activities decreased dramatically on slicing turnip, swede, and beetroot tissues (Fig. 1). While minimum activities were usually detected 3–5 hr after slicing, most of the loss occurred in the first 10 min. Subsequent to the decline, activities generally increased with further aging. Maximum NADH-cytochrome  $c$  reductase activity developed by about 24 hr, after which activity again decreased, but at a much slower rate than that initiated by slicing. Microsomal

protein and NADH-ferricyanide (FeCN) and NADH-2,6-dichlorophenolindophenol (DCPIP) reductase levels continued to increase after this decline in NADH-cytochrome *c* reductase. Activities in Figure 1 are presented on a protein basis to demonstrate that the induced changes were in character rather than yield (the loss of protein induced immediately by slicing may be a result of less efficient homogenization of disks compared with whole tissue). The cytochrome *b*<sub>3</sub> level in the turnip microsome fraction paralleled the NADH-cytochrome *c* reductase activity.

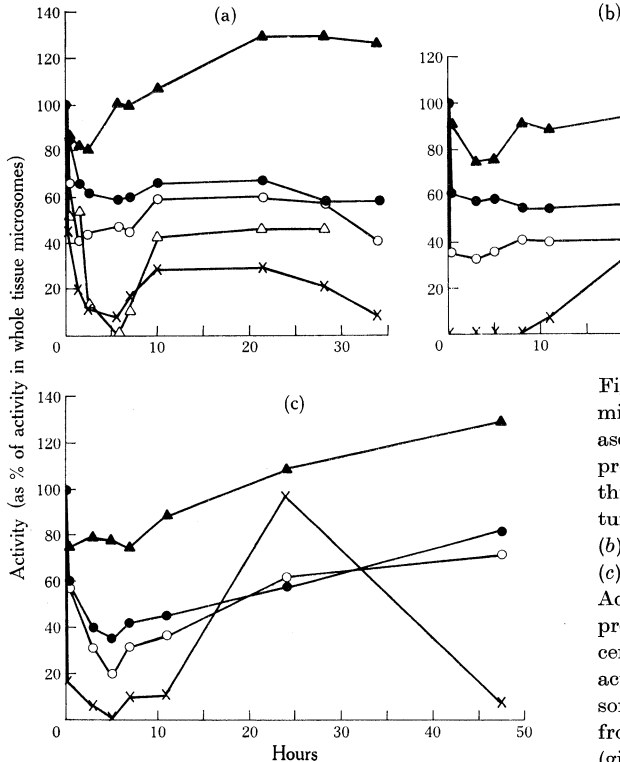


Fig. 1.—Changes in microsomal reductase activities and protein in 1 mm thick disks of turnip (a), swede (b), and beetroot (c) with aging. Activities are expressed as a percentage of the activities in microsomes isolated from whole tissue (given below in

μmoles NADH oxidized/min/mg protein): NADH-cytochrome *c* (×): (a), 0.273; (b), 0.045; (c), 0.078. NADH-DCPIP (○): (a), 0.393; (b), 0.232; (c), 0.426. NADH-FeCN (●): (a), 0.900; (b), 0.320; (c), 1.18. Cytochrome *b*<sub>3</sub> (Δ): (a), 0.35 nmoles/mg protein. ▲ Protein.

(b) *Relationship between Induced Microsomal Changes and RNA and Protein Synthesis in Turnip Disks*

Figure 2(a) shows that cycloheximide ( $10^{-5}$ M) had no effect on the loss of microsomal activities induced by slicing, but inhibited the subsequent increases. Activity levels in disks aged for 12 hr in the presence of cycloheximide were similar to the minimum levels of activity detected in the absence of inhibitor (Table 1). The minimum level of activities detected after slicing (Fig. 1) cannot, therefore, be due to a balance between inactivation and synthesis. However, 6-methylpurine (at a concentration which did not inhibit respiration—Gayler and Glasziou 1968) only partially

inhibited reductase development (Table 1). Hence the increases in microsomal reductase activities were dependent on protein synthesis but only partially on RNA synthesis.

The net activities measured at various times after slicing were a resultant of the rate of loss and rate of synthesis. An indication of the rate of breakdown could be determined by adding cycloheximide to the disks after resynthesis had become apparent and then assaying for any subsequent loss of activity in the absence of further synthesis. Addition of cycloheximide after aging for 8 hr inhibited further

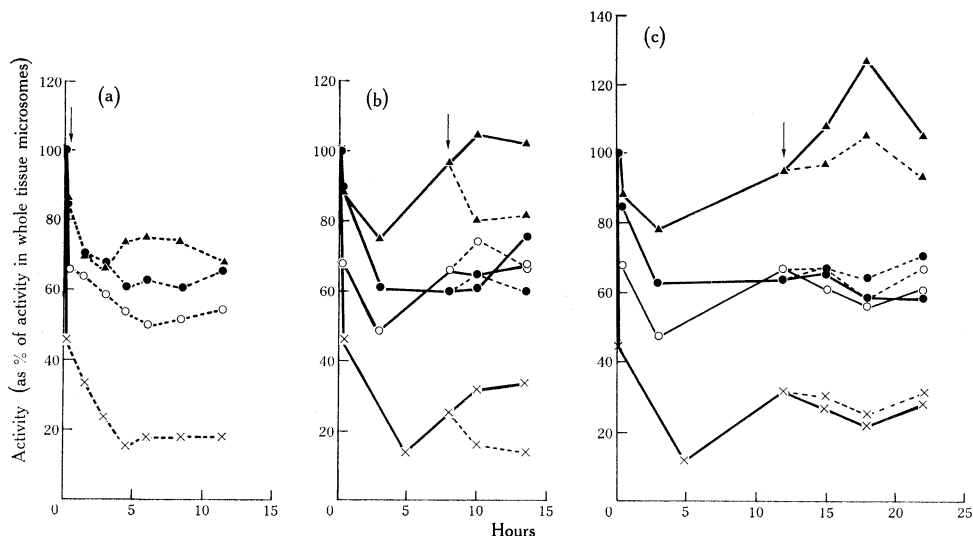


Fig. 2.—Effect of addition of cycloheximide to aging turnip disks at 0 (a), 8 (b), and 12 (c) hr after slicing on microsomal reductase activities and protein. Addition of cycloheximide ( $10^{-5}$ M) is indicated by the arrows. Disks aged in the absence of cycloheximide are represented by solid line and in presence of cycloheximide by broken line. Activities are expressed as a percentage of the activities in microsomes isolated from whole tissue (given below in  $\mu$ moles NADH oxidized/min/mg protein): NADH-cytochrome *c* (x): (a), 0.115; (b), 0.140; (c), 0.124. NADH-DCPIP (o): (a), 0.440; (b), 0.460; (c), 0.445. NADH-FeCN (●): (a), 0.839; (b), 1.31; (c), 1.03. ▲ Protein.

NADH-FeCN and NADH-DCPIP reductase increase, but initiated a rapid loss of NADH-cytochrome *c* reductase and of microsomal protein [Fig. 2(b)]. This rapid drop in microsomal protein had the effect of inflating the activities (on a protein basis) in the microsome fractions isolated from disks aged in cycloheximide relative to the control. However, per gram fresh weight of tissue, the NADH-FeCN and NADH-DCPIP reductase activities remained at the level when cycloheximide was added, while the drop in NADH-cytochrome *c* reductase was greater than indicated in Figure 2(b). Cycloheximide added at 12 hr had no effect on reductase activities or protein levels, other than to inhibit further syntheses [Fig. 2(c)]. This indicated that 8 hr after slicing NADH-cytochrome *c* reductase and protein losses were still occurring, although rate of synthesis was sufficiently great for this not to be apparent. However, by 12 hr the inactivating mechanism had been eliminated.

*(c) Effect of Disk Size on Induced Microsomal Changes*

The large loss of NADH-cytochrome *c* reductase activity observed on slicing cannot be attributed to the small percentage of damaged cells on the surface of the disks [Fig. 1(a)], suggesting that a stimulus was transmitted to the inner cells. Repeating this experiment with disks 10 mm thick resulted in no significant differences in the rate of breakdown, levels of minimum activity or rate of initial resynthesis (Fig. 3). Hence such a stimulus was equally effective in disks 10 and 100 cells thick. However, after aging for 8 hr there was a sudden decline in all reductase activities,

TABLE 1

EFFECT OF CYCLOHEXIMIDE AND 6-METHYLPURINE ON THE DEVELOPMENT OF MICROSOMAL ACTIVITIES IN AGING TURNIP DISKS

Inhibitors were added immediately after slicing and disks harvested after aging for 12 hr. All activities (except respiration) are expressed as a percentage of the activities per milligram protein in microsomes isolated from whole tissue. Respiration is expressed as a percentage of the rate in fresh disks

Activity	Cycloheximide concn. (M)				6-Methylpurine concn. (M)			
	0*	0	$2.5 \times 10^{-6}$	$10^{-5}$	0*	0	$10^{-5}$	$5 \times 10^{-5}$
NADH-cytochrome <i>c</i> reductase	8	22	16	9	8	50	35	21
NADH-DCPIP reductase	44	68	50	51	41	102	91	76
NADH-FeCN reductase	52	76	56	59	59	90	70	81
Protein	76	96	76	78	81	94	83	81
Cytochrome <i>b</i> <sub>3</sub>	17	50	33	17				
Respiration						115	100	68

\* These disks aged for 3–5 hr. All other disks aged for 12 hr.

particularly NADH-cytochrome *c* reductase, in 10-mm thick disks. This apparent inhibition, from which there was no recovery with further aging, resembled the effect of cycloheximide added at 8 hr [Fig. 2(b)]. Corresponding to this Laties (1967) showed that respiration was independent of potato disk thickness (to 3 mm) for the first 8 hr after slicing. He suggested that respiration was under the control of a diffusable metabolite (rather than oxygen tension) which probably inhibited both RNA and protein synthesis. It was released on slicing but built up again in the inner cells of thicker disks.

*(d) Effect of Indoleacetic Acid (IAA) on Microsomal Activities with Aging*

Palmer (1970) reported an IAA-induced acid phosphatase release from jerusalem artichoke slices, possibly as a result of change in permeability of the lysosome membrane. Turnip disks were aged in the presence of IAA to test if there was any effect on microsomal breakdown and resynthesis that might be correlated with the suggested increased lysosomal activity. Table 2 shows that at low concentrations ( $6 \times 10^{-6}$ M), IAA caused a specific decrease in NADH-cytochrome *c* reductase activity,

not accompanied by losses of any other parameters. Higher concentrations resulted in a decrease in microsomal protein and of the other reductase activities. This may be due to increased destruction of the enzymes or of the messenger RNA required for

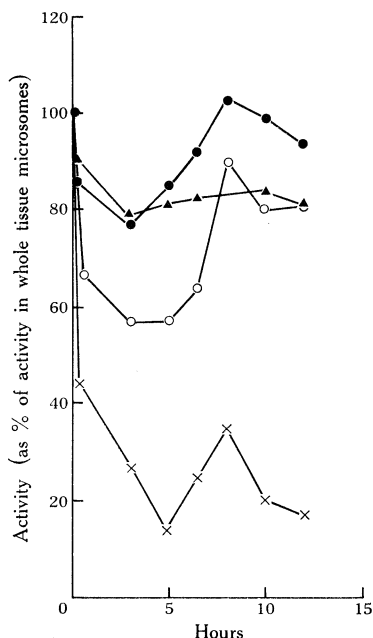


Fig. 3.—Changes in microsomal reductase activities and protein in 1 cm thick turnip disks with aging. Activities are expressed as a percentage of the activities in microsomes isolated from whole tissue (given below in  $\mu$ moles NADH oxidized/min/mg protein): NADH-cytochrome *c* (x) 0.159; NADH-DCPIP (o) 0.413; NADH-FeCN (●) 0.929. ▲ Protein.

production of the enzymes or both (Glasziou, Waldron, and Bull 1966). As respiration was unaffected, this effect can presumably not be attributed to cellular degradation.

TABLE 2

## EFFECT OF INDOLEACETIC ACID ON MICROSOMAL ACTIVITIES IN AGING TURNIP DISKS

Indoleacetic acid was added immediately after slicing and disks harvested after aging for 8 hr. All activities (except respiration) are expressed as a percentage of the activities in microsomes isolated from whole tissue. Respiration is expressed as a percentage of the rate in fresh disks

Activity	Indoleacetic acid concentration (M)					
	0*	0	$10^{-6}$	$6 \times 10^{-6}$	$6 \times 10^{-5}$	$3 \times 10^{-4}$
NADH-cytochrome <i>c</i> reductase	8	28	31	18	17	17
NADH-DCPIP reductase	41	49	52	55	45	38
NADH-FeCN reductase	59	54	63	58	55	46
Protein	81	107	119	110	109	81
Respiration		127	148	121	130	134

\* These disks aged for 3–5 hr. All other disks aged for 8 hr.

Figure 4 shows the effect of IAA ( $3 \times 10^{-4}$ M) on the microsomal activity changes in aging disks under the conditions used by Palmer (1970) to induce maximum

phosphatase activity (i.e. without  $\text{CaSO}_4$ ). Loss of NADH-cytochrome *c* reductase was greater, and of NADH-FeCN and NADH-DCPIP reductases less, in IAA than in the control. Decrease in rate of loss of the latter activities was a result of the accompanying high loss of protein [Fig. 4(*d*)]. IAA also prevented the subsequent increases in activities. Hence there does appear to be a correlation between reductase losses and the IAA-induced hydrolytic activity (Palmer 1970).

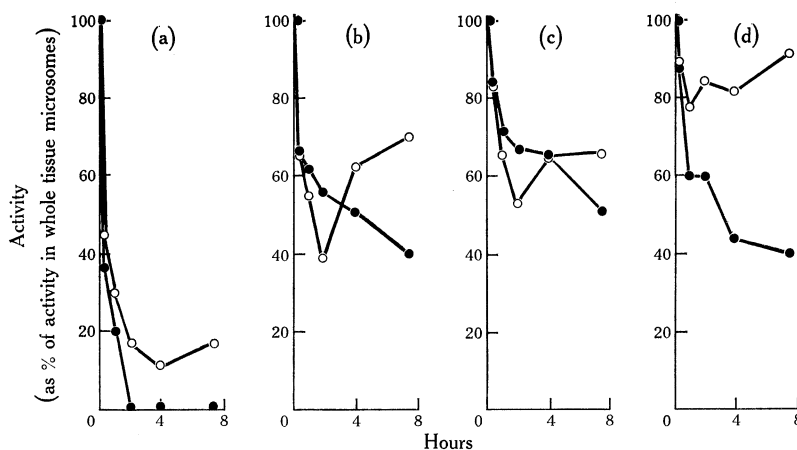


Fig. 4.—Effect of aging turnip disks in the absence (○) and presence (●) of  $3 \times 10^{-4}\text{M}$  indoleacetic acid on microsomal reductase activities (*a-c*) and protein (*d*). Activities are expressed as a percentage of the activities in microsomes isolated from whole tissue (given below in  $\mu\text{moles NADH oxidized/min/mg protein}$ ): (*a*), NADH-cytochrome *c* 0.264 (○), 0.174 (●); (*b*), NADH-DCPIP 0.340 (○), 0.367 (●); (*c*), NADH-FeCN 0.715 (○), 0.907 (●).

The effect of  $10^{-4}\text{M}$   $\text{CaSO}_4$  on the action of  $3 \times 10^{-4}\text{M}$  IAA can be considered by comparing microsomal activities after aging for 8 hr in the absence (Fig. 4) and presence (Table 2) of  $\text{CaSO}_4$ .  $\text{CaSO}_4$  protected against losses of NADH-cytochrome *c* reductase activity and protein while having no effect on the NADH-DCPIP and NADH-FeCN reductase losses. Van Steveninck (1965) showed that IAA caused leakage of ions from beet cells but that the effect was not reversed by  $\text{CaSO}_4$ . This may suggest that the IAA effect on microsomal reductases was not related to the cell permeability changes reported by Van Steveninck.

#### (e) Characterization of the Induced Losses of Microsomal Activities

The decrease in reductase activities, not accompanied by an equivalent decrease in protein, could be best explained by a rapid release of hydrolytic enzymes. These may permeate from the sliced cells into the neighbouring intact cells or may actually be released by the intact cells (due to a permeability change). In contrast to animal cells, and meristematic and differentiating plant cells, most of the hydrolytic enzymes are likely to be in the large central vacuole of mature plant cells (Matile and Moor 1968), and therefore liberated into the soluble supernatant fraction on homogenization. Hence, the effect on the reductase activities of incubating isolated microsomes

with the soluble fraction was determined (Fig. 5). It was not possible to measure release of reductase components from the membranes to the soluble fraction, due to interference from other soluble reductases and peroxidase. Hence activity losses could only be detected by assaying the microsomes after separation from the soluble fraction. As there appeared to be no *in vivo* losses of the microsomal reductase activities after aging for 12 hr [Fig. 2(c)], the effects of soluble fractions on microsomal fractions isolated from both fresh disks and disks aged for 12 hr were determined. Incubation with the soluble fraction did cause up to 60% loss of microsomal reductase activities. However, the soluble fraction from aged disks inhibited the microsomal

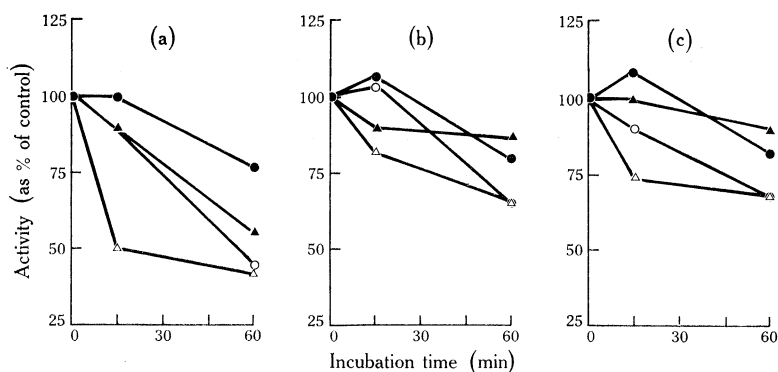


Fig. 5.—Effect of incubating turnip microsomes from fresh (open symbols) and 12 hr aged (closed symbols) tissue with the soluble supernatant fraction from fresh (circles) and 12 hr aged (triangles) tissue on the reductase activities. Microsomes from approximately 70 g tissue were incubated with 30 ml of undiluted supernatant [previously adjusted to pH 7.2 with 1M tris(hydroxymethyl)-amino methane] in the presence of 70 mM Tris-HCl (pH 7.2), at 25°C with constant shaking. After 15 and 60 min aliquots of the incubation mixture were chilled, centrifuged at 54,000 *g* for 60 min, and the supernatant discarded. The pellet was resuspended in 0.4M sucrose, recentrifuged, and the resulting pellet resuspended in the same initial volume of 0.4M sucrose (2.0 ml). Corrections have been made for the losses of activity in the controls. Activities are expressed as a percentage of activities in the untreated microsomes (given below in  $\mu$ moles NADH oxidized/min/mg protein): (a), NADH-cytochrome c 0.035 (fresh), 0.029 (aged); (b), NADH-DCPIP 0.102 (fresh), 0.102 (aged); (c), NADH-FeCN 0.313 (fresh), 0.265 (aged).

reductases more (rather than less) than the soluble fraction from fresh tissue. (The inactivating mechanism *in vivo* was presumably not controlled by a rapid turnover, as storage of the soluble fraction for 48 hr at 4°C did not result in any loss of its inhibitory activity.) However, the microsomal reductases from aged disks were all significantly less sensitive to the soluble fractions than those from fresh tissue (Fig. 5).

#### IV. DISCUSSION

##### (a) Correlation with Reported Biochemical Changes in Microsomes with Aging

The rapid synthesis of microsomal phospholipids (and presumably membranes) for approximately 8 hr after slicing (Tang and Castelfranco 1968) correlated with the period of greatest increase in microsomal protein and NADH-DCPIP and NADH-FeCN reductases. This also corresponded with the period of highest protein synthesis



in aging disks (Kahl, personal communication). Hackett *et al.* (1960) and Ben Abdelkader (1969) both reported four- to sevenfold increases in microsomal reductases after aging potato disks for 24 hr. However, in both cases the comparison was between fresh and aged disks, i.e. their controls were the low inactivated levels of fresh disks rather than the higher levels of whole tissue. The equivalent NADH-cytochrome *c* reductase activities reported by Ben Abdelkader (1969) in fresh disks and disks aged for 24 hr in cycloheximide may be explained by the low concentration of inhibitor used ( $1.8 \times 10^{-6} \text{M}$ ). Table 1 shows that a concentration slightly higher than this only partially inhibited the subsequent increase in NADH-cytochrome *c* reductase and cytochrome *b*<sub>3</sub> in aging turnip disks.

*(b) Correlation with Reported Ultrastructural Changes in Endoplasmic Reticulum with Aging*

As most of the activity losses occurred immediately on slicing, any associated ultrastructural changes would occur when whole tissue was cut for fixing. Hence these changes would not be detected and the resulting structure of the endoplasmic reticulum would be interpreted as the natural state in intact tissue. However, slicing tissue into disks induced no change in the endoplasmic reticulum of swede (Van Steveninck 1970) but induced vesiculation in the endoplasmic reticulum of beet (Jackman and Van Steveninck 1967). Correlated with these observations, there were further losses of reductase activities (after the initial losses) in beet but not swede (Fig. 1). The endoplasmic reticulum strands in beetroot reformed and increased with further aging (Jackman and Van Steveninck 1967) and there was also an overall increase in the endoplasmic reticulum of jerusalem artichoke (Fowke and Setterfield 1968), but not of swede or carrot (Van Steveninck 1970). The increases in microsomal protein and NADH-FeCN and NADH-DCPIP reductases in beet but not in swede disks with further aging is in agreement with these ultrastructural changes.

Hence there appeared to be a correlation between changes in NADH-DCPIP and NADH-FeCN reductases and the state of the endoplasmic reticulum. This correlation did not always hold for total protein or for NADH-cytochrome *c* reductase which, unlike the other reductase activities, involves the microsomal cytochrome *b*<sub>3</sub> (Rungie and Wiskich 1972). The microsomal cytochrome is loosely attached to the outside of the membrane and is readily solubilized (Takesue and Omura 1970). Therefore loss of NADH-cytochrome *c* reductase activity (by solubilization of cytochrome *b*<sub>3</sub>) would precede other *in vivo* losses and would not result in any observable ultrastructural change.

Development of endoplasmic reticulum (Van Steveninck 1970) and microsomal reductases were both prevented by protein synthesis inhibitors. While RNA synthesis was not involved in the development of the endoplasmic reticulum (Van Steveninck 1970), it was partially involved in the development of the reductases (Table 1). Hence endoplasmic reticulum development may continue without the full complement of microsomal reductases. Van Steveninck (1970) suggested a lack of direct correlation between increase in endoplasmic reticulum and development of the ion accumulation process in aging beet disks as the latter was sensitive to actinomycin. However, such a correlation may still exist as the development of ion accumulation would be dependent

on the electron transport enzymes of the endoplasmic reticulum rather than the membrane itself.

(c) *Nature of the Induced Microsomal Activity Losses*

The rapidity of microsomal activity loss on tissue slicing indicated that it was not due to cessation of synthesis in the normal turnover of enzymes (the half-lives of the corresponding animal enzymes were about 3 days, Kuriyama *et al.* 1969). The specificity of the losses indicated that non-specific osmotic damage was not likely to be involved (Kahl, personal communication). (Also, similar results were obtained if the turnip tissue was sliced and aged in isotonic sucrose.) The *in vivo* effect of IAA (Fig. 4) and *in vitro* effect of the soluble fraction (Fig. 5) on the microsomal reductases suggest the action of hydrolytic enzymes, although it is not clear whether this results in direct hydrolysis of the microsomal membranes (Takesue and Omura 1970), release of inhibitory fatty acids (Dalgarno and Birt 1963; Galliard 1970), or both. However, the correlation between ultrastructural change in endoplasmic reticulum and the change in microsomal reductases indicates hydrolysis of membranes rather than enzyme inhibition. Jones and Wakil (1967) demonstrated a phospholipid requirement for liver microsomal NADH-cytochrome *c* but not NADH-FeCN reductase activity. Hence, hydrolysis of phospholipids in tissue slices may result in some loss of microsomal activities, and ultrastructural change of the endoplasmic reticulum. The rapid synthesis of microsomal phospholipids for 8 hr after slicing (Tang and Castelfranco 1968) may reverse these effects.

It is also possible that reductase components are actually released from the membranes. An NADH-FeCN reductase in plant-soluble (unpublished observation) and *b*-type cytochromes in plant- and animal-soluble supernatant fractions have been detected (Shichi and Hackett 1966; Mangum, Klingler, and North 1970). While these resemble the corresponding microsomal components, it is not known whether they are derived from the microsomal membranes. Slicing does induce a loss of microsomal protein which parallels reductase loss but this may not indicate specific solubilization as the two effects were shown to be only partially related [Fig. 2(b)].

The addition of cycloheximide to disks immediately after slicing resulted in loss of microsomal reductases to a constant level [Fig. 2(a)]. Similar partial insensitivity of the reductases to various disruptive treatments of the isolated microsomes has also been observed (Rungie and Wiskich 1972). Ito and Sato (1969) showed that a bacterial protease acted on only the surface of liver microsomal membranes resulting in a structure insensitive to further hydrolysis. In tissue aged for 12 hr there was no loss of reductase activities [Fig. 2(c)], indicating inhibition of the inactivating mechanism and possibly resynthesis of an insensitive endoplasmic reticulum structure. *In vitro*, the reductases of the microsomes prepared from aged tissue were less sensitive to incubation with the soluble fraction than those prepared from fresh tissue possibly as a result of increased stability due to increased phospholipid (Tang and Castelfranco 1968). However, as this effect was incomplete, other factors must also be involved *in vivo*.

It would be surprising if other membranes were not affected by slicing in a similar manner to the endoplasmic reticulum. However, Jackman and Van Steveninck

(1967) noted no significant changes in the appearance of other organelles. There is increasing evidence that the outer mitochondrial and microsomal membranes in animals are very similar (Sottocasa *et al.* 1967), although there are some marked differences in trypsin sensitivity (Kuylenstierna *et al.* 1970). Hackett *et al.* (1960) reported a 10-fold increase in the antimycin A-insensitive NADH-cytochrome *c* reductase activity in potato disks after aging for 24 hr. This resembled the increase in NADH-cytochrome *c* reductase activity in the microsome fraction of the same (Hackett *et al.* 1960) and other tissues (Fig. 1).

#### V. ACKNOWLEDGMENTS

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