Relationship of Lipid Metabolism to the Respiration and Growth of Pea Stem Sections

David Penny and Bruce B. Stowe

Department of Biology, Yale University, New Haven, Connecticut

Received July 19, 1965.

Summary. Biologically active lipids increase the growth of pea stem sections within 3 hours at the same time their respiration is increased and their growth rate is more than that of the intact plant. The greater final length of the intact internode is due to a longer growth period.

Both active and inactive lipids are rapidly taken up and enter all major metabolic fractions; among centrifugal fractions methyl oleate tends to label those that contain metabolically active membranes. It is concluded that lipids active in the bioassay are probably the effective molecules at the subcellular site of action.

No direct effect of lipids on isolated mitochondria could be shown. The respiration of stem tissue was not influenced by dinitrophenol and carbonyl cyanide m-chlorophenyl hydrazene although dinitrophenol inhibited growth. Lipid-induced respiration was sensitive to these agents as well as to cyanide, indicating cytochrome oxidase is probably involved.

The promotion of growth and respiration by lipids is not linked to protein synthesis, since actinomycin D, puromycin and cycloheximide failed to inhibit the respiratory increase even though strongly limiting amino acid incorporation into protein. It is most likely that the effect of lipids on growth is due to their promotion of respiration.

A large variety of lipids in comparatively low concentrations will increase the growth of certain pea stem sections although the growth is still less than the intact stem (22, 24). At least 4 representatives of the classes of lipids that increase growth also increase respiration at the same concentrations and under the same experimental conditions (18) and it seems likely that there is a causal connection between the effects of lipids on growth and on respiration.

The mechanism of action of the lipids on growth or respiration is not yet known. Very little is known about the biochemical mechanisms by which growth substances increase the elongation of stems, whereas the regulation of respiration is becoming increasingly well understood. For this reason the lipid effect on respiration has been studied in more detail in this paper since, if the effect on respiration is understood, it may help explain the effect of lipids on growth.

Materials and Methods

Pea seeds (Pisum sativum L.) of the dwarf variety Alaska were grown as described in an earlier publication (24) in vermiculite and under a weak red light source (0.1 ergs/cm² per sec between 600-800 m/). Sections 10 mm long were cut under a green safelight from third internodes that were between 15 and 30 mm long. The basal bioassay medium used contained 1.5% sucrose, 5 mM KH₂PO₄ (pH 5.5), 50 µM CoCl₂, 1.8 µM IAA, 0.3 µM GA₃ and 0.004% of the emulsion stabilizer Pluronic F68 (22).

Seeds of the tall variety Alaska were grown in vermiculite under a mixed incandescent-fluorescent light source of about 1500 ft-c (19). Ten mm sections were cut 1 mm below the top of the fourth internode from plants whose fifth internode was about 5 mm long. Five sections were placed in 5 cm diameter petri dishes containing 5 ml of a solution of 1% sucrose, 10⁻⁴ M IAA and 0.025 M phosphate buffer (pH 6.1) (19). Ten mm segments on the intact plant were marked with a mixture of lanolin and charcoal and the distance between the marks measured at various times thereafter.

Triolein-1-C¹⁴ and methyl oleate-U-C¹⁴ were ob-

---

1 This investigation was supported in part by Public Health Service Research Grant GM-06921 from the National Institutes of Health.
2 Present address: Research Institute in Biochemistry, McMaster University, Hamilton, Ontario, Canada.
tained from Applied Science Laboratory Inc., State College, Pennsylvania and oleic acid-1-C\textsuperscript{14} from Calbiochem Corporation. These were routinely purified by thin layer chromatography before use (16). It is important that compounds be checked for purity since some lipid samples were found to be grossly impure.

Lipids were extracted from plant material by a procedure modified from that of Kates and Eberhardt (13, Obreiter unpublished). After the sections were rinsed they were extracted and washed first in boiling isopropanol and then in a 50:50 mixture of boiling isopropanol:chloroform. After a wash in boiling chloroform all the filtrates were pooled and concentrated at 40° on a rotary evaporator. This was made up to 50 ml with chloroform, shaken with 50 ml of water and centrifuged to separate the 2 layers. The radioactivity in the aqueous layer was counted and the chloroform layer containing lipids fractionated into neutral and phospholipid fractions by shaking with 200 mesh silicic acid before being filtered and washed with chloroform. The nonabsorbed neutral lipids were retained in the chloroform washings. The phospholipid fraction was absorbed on the silicic acid and was removed with methanol.

To examine the distribution of radioactivity among different centrifugal fractions the tissue was washed after exposure to C\textsuperscript{14}-methyl oleate and ground in 0.3 m sucrose and 0.02 m phosphate buffer pH 7.2. The residue that did not pass through 4 layers of cheesecloth was retained and the sediments collected at 1000, 12,000 and 100,000 × g. The final supernatant fraction was counted directly; the other fractions were freeze dried before extractions for 4 hours by chloroform with acetone or chloroform in a Soxhlet apparatus. Additional extractions with acetone or chloroform removed little additional radioactivity.

A mitochondrial fraction was isolated by grinding the sections with a pestle and mortar for 5 minutes in at least 5 times their weight of modified Honda medium (12).

The medium consisted of 1.0 mm manganese acetate, 0.3 m sucrose, 25 g/liter Ficoll (Pharmacia Labs Inc., Rochester, Minnesota), 1.0 mm magnesium acetate, 3.0 mm GSH, 0.01 m EDTA, 0.05 m Tris, pH 7.8, 100 mg/liter bovine serum albumin.

While the tissue was being ground the pH was followed using a narrow range pH paper and adding KOH to maintain the pH between pH 6.5 and 8.0, preferably between pH 7.0 and 7.5 (26). The homogenate was passed through 4 layers of cheesecloth and centrifuged at 1000 × g for 10 minutes. This supernatant fraction was centrifuged at 5000 × g for 20 minutes and resuspended in an unbuffered 0.3 m sucrose and 1.0 mm magnesium sulfate solution between pH 7.0 and 7.5. This was centrifuged again at 5000 × g and resuspended.

Phosphorylation was measured by removing 0.5 ml of the medium containing the mitochondria from the Warburg vessels and adding it to 2 ml of cold 10% trichloroacetic acid and centrifuging. Inorganic phosphate was determined by reducing the phosphomolybdate complex with ascorbic acid (6).

Measurements of O\textsubscript{2} consumption on sections and cell fractions were made with a Warburg manometer as described previously (18). Cyanide was used according to the directions of Robbie (20).

To test the effectiveness of the antibiotics used to stop protein synthesis, the incorporation of C\textsuperscript{14} leucine into protein was studied. The sections were pretreated in the antibiotic for 30 minutes. The incorporation of counts into a protein fraction that was insoluble in 80% ethanol (v/v); 5% trichloroacetic acid; and in 3:1 ethanol: ether (17) was measured in a scintillation counter.

**Results**

**Kinetics.** The time course of elongation of the marked zone on the intact stem is compared to that of the sections with or without triolein (fig 1). A triolein-induced increase in the elongation of the sections can be detected after 3 hours, which is the same time that increased respiration and elongation was found with sections in the Warburg vessels (18). A stimulation has been observed after 2 hours in an experiment when the first measurement was made at this time. Thus, the lipid effect on growth occurs at the same time that respiration is increased.

These results indicate that the elongation of the sections can exceed that of the intact stem over the first few hours. Whether or not the sections exceed the growth of the intact stem depends on the time of measurement.

With green Alaska peas it has also been shown that the sections can exceed the growth of the stems

![Fig. 1. Kinetics of growth of 10 mm sections and 10 mm zone of intact stem of red light-grown Progress no. 9 peas. Sections grown in basal medium (table II), 18 ppm IAA, 0.3 ppm GA<sub>3</sub> with or without 20 µM triolein.](image-url)
Table I. Comparison of Growth of Sections with Intact Stem Zones of Green Alaska Peas

Increase in length and of fresh weight of 10 mm sections or zones measured after 20 hours. Average of 70 plants per treatment. Sections incubated in 1% sucrose, 10^{-4} M IAA, 0.025 M KH_{2}PO_{4} buffer, pH 6.1.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Sections</th>
<th>Increase in length mm/plant</th>
<th>Intact zone</th>
<th>Increase in fr wt mg/plant</th>
<th>Intact zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.8</td>
<td>8.4</td>
<td>18.1</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.8</td>
<td>6.9</td>
<td>18.0</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.8</td>
<td>5.6</td>
<td>21.6</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9.9</td>
<td>6.4</td>
<td>16.6</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Avg</td>
<td>9.8</td>
<td>6.9</td>
<td>18.8</td>
<td>13.1</td>
<td></td>
</tr>
</tbody>
</table>

Even though the sections were yellowish after 24 hours and were found to contain 2.5 times less chlorophyll per segment than the intact plant.

Metabolism of the Lids. It is important to know whether the active molecules in vivo are the same compounds that are active when added in the bioassay. If the exogenous lipids must be metabolized by the tissue before they are active then it may not be valid to look for the effect of, for example, triolein, on a mitochondrial fraction. It was possible that the active molecules in vivo were phospholipids since preliminary experiments had shown that significant amounts of C^{14} was recovered in this fraction after adding C^{14}-methyl oleate to the tissue.

The most successful approach to determine whether the exogenous lipids need to be metabolized has been to compare the uptake and metabolism of molecules which are active and inactive in the bioassay. Oleic acid is inactive in this bioassay (23) although it is converted into phospholipids. However, the inactivity of oleic acid could have been because it was only slowly taken up and metabolized by the tissue. For this reason the uptake and metabolism of oleic acid was compared with 2 active compounds, methyl oleate and triolein (table II).

The aqueous phase comes from the fractionation of the isopropanol chloroform extract between aqueous and chloroform phases. The method used for counting C^{14}O_{2} was described previously (18).

These results show that oleic acid is metabolized more readily than either methyl oleate or triolein. Hence, the inactivity of oleic acid is not due to its failure to be metabolized, since it as well as the active lipids are found to enter all major metabolic fractions. Also, it is probable that the effective added lipids are the active molecules per se, rather than some metabolite derived from them.

Subcellular Fractions. The recovery of radioactivity from methyl oleate among subcellular fractions is shown in table III. As expected, the radioac-

Table II. Relative Distribution of C^{14} in Biochemical Fractions after Incubation of Pea Stem Sections with Labeled Lipids

10 mm Progress no. 9 sections incubated for 4.5 hours in basal medium [1.5% sucrose, 5 mM KH_{2}PO_{4} (pH 5.5), 50 μM CoCl_{2} and 0.004% Phlorhizin F-68]. 1.8 μM IAA, 0.3 μM GA_{3} and lipid.

<table>
<thead>
<tr>
<th>% radioactivity recovered in each fraction</th>
<th>Oleic acid: U-C^{14}</th>
<th>Methyl oleate: U-C^{14}</th>
<th>Triolein: L-C^{14}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>20 μM</td>
<td>50 μM</td>
<td>20 μM</td>
</tr>
<tr>
<td>Neutral lipid</td>
<td>7.5</td>
<td>2.9</td>
<td>5.4</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>5.0</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Aqueous phase</td>
<td>28.0</td>
<td>1.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>1.5</td>
<td>0.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Tissue after extraction</td>
<td>3.1</td>
<td>1.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Other</td>
<td>0.3</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Total in tissue</td>
<td>45.4</td>
<td>8.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Left in bioassay medium</td>
<td>53.6</td>
<td>91.0</td>
<td>93.4</td>
</tr>
<tr>
<td>Total recovered</td>
<td>99.0</td>
<td>99.2</td>
<td>99.7</td>
</tr>
</tbody>
</table>

Table III. Distribution of C^{14} Among Centrifugal Fractions after Incubation of Pea Stem Sections with Methyl Oleate-1-C^{14}

10 mm Progress no. 9 sections incubated for 4.5 hours in basal medium, IAA, GA_{3} (as in table II) and 50 μg lipid.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Cpm</th>
<th>Dry wt g</th>
<th>Cpm/mg Dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue</td>
<td>59,000</td>
<td>1.00</td>
<td>59</td>
</tr>
<tr>
<td>1000 × g</td>
<td>1025</td>
<td>0.021</td>
<td>51</td>
</tr>
<tr>
<td>12,000 × g</td>
<td>11,200</td>
<td>0.028</td>
<td>397</td>
</tr>
<tr>
<td>100,000 × g</td>
<td>13,200</td>
<td>0.017</td>
<td>778</td>
</tr>
<tr>
<td>Supernatant</td>
<td>1125</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Activity is accumulated in fractions richest in metabolically active membranes. The large amount of radioactivity in the residue that did not pass through the cheesecloth may be due to incomplete isolation of mitochondria and other particles.

It has been estimated (11) that this method of isolation yields only 25% of the mitochondria of the tissue.

Effect of Triolein on Mitochondria. The molecules that are active in this bioassay have a very similar chemical specificity to those lipids that reactivate isooctane extracted mitochondria (23). For this reason it was suggested that in these sections the exogenous lipids acted directly on the mitochondria to increase respiration and that this increased growth (23). A mitochondrial fraction has been isolated but no effects of triolein on either phosphorylation or O_{2} consumption have been found. An example of the data obtained is shown in table IV. The reduction of P:O at 2 μM triolein is probably not real since it is absent at 20 μM triolein and because of the variability between experiments. Other substrates that have been tried are α-ketoglutaric, malic and iso-
citric acids, and succinic acid has been tested in the absence of added ADP and without the glucose hexokinase trap.

**Uncoupling Agents.** Because no effects of triolein have been found on isolated mitochondria, the respiration of the sections has had to be studied in more detail. If phosphorylation is limiting respiration, and if triolein increases respiration by an increase in the levels of phosphate acceptors, then uncoupling agents should increase \( O_2 \) consumption to at least the same extent as triolein. Table V shows the results obtained with 2,4-dinitrophenol (DNP) and carbonyl cyanide m-chlorophenyl hydrazine (CCCP) (7). Neither gives any significant stimulation in the absence of triolein. The slight stimulation by DNP is probably not real since there was a slight inhibition at this concentration in another series of experiments. DNP has been tested from \( 3 \times 10^{-6} \) to \( 10^{-3} \) M without finding a stimulation at any concentration. The concentrations of CCCP used varied between \( 10^{-3} \) and \( 10^{-2} \) M. Triolein gave the usual stimulation in these experiments but in the presence of triolein the uncoupling agents increased respiration to even higher levels.

There are several possible interpretations for the lack of effect of uncoupling agents unless triolein is present. It was possible that uncoupling agents did not penetrate the sections unless the lipids were present. However, the effect of DNP on fresh weight was measured on the same sections (table V) and even in the absence of the triolein DNP does inhibit growth, presumably by uncoupling respiration. The respiration of sections from the first internode was stimulated by DNP and it is known that sections further down the plant show a bigger response to DNP (1). Thus, it is unlikely that the lack of effect of uncoupling agents is due to an inability to penetrate the tissue in the absence of triolein.

It is possible that the increased \( O_2 \) consumption does not go through the usual cytochrome oxidase, but through some other terminal oxidase which is not affected by phosphorylation. Some evidence against this possibility is that DNP does increase respiration when triolein is present, which suggests that the triolein-induced respiration is mediated by cytochrome oxidase. More direct evidence comes from the use of cyanide to inhibit respiration. Sections respiring at \( 330 \mu \)l \( O_2/g \) fresh weight per hour were stimulated to 446 \( \mu \)l \( O_2/g \) fresh weight per hour by 20 \( \mu \)M triolein, yet \( 10^{-3} \) M cyanide reduced both untreated and lipid treated to the same basal level of 39 and 37 \( \mu \)l \( O_2/g \) fresh weight per hour respectively. Thus, the triolein-induced component of respiration is cyanide sensitive.

**Relation to Protein Synthesis.** Another alternative which has been considered is that triolein increased the synthesis of enzymes that were limiting respiration. In this case, stopping RNA and protein synthesis by antibiotics should prevent the triolein-induced respiration. Neither actinomycin D at \( 10 \mu g/ml \) puromycin at \( 10^{-2} \) M, or \( 10 \mu g/ml \) cycloheximide had any influence on \( O_2 \) consumption or upon its rise in the presence of 20 \( \mu \)M triolein. The antibiotics did succeed in hindering protein synthesis, since 5 \( \mu l \) of L-leucine-U-C\(^{14}\) (220 c/mole), added 30 minutes after the antibiotic and incubated 30 minutes, was taken up equivalently into all sections, but its incorporation into protein was cut to 50% by puromycin and to 7% by cycloheximide. The respiratory increase is thus independent of protein synthesis, and probably of RNA metabolism.

**Discussion**

The higher growth rate induced by lipids occurs fairly uniformly through the period of elongation,

---

**Table IV. Effect of Pluronic F-68 and Triolein on \( O_2 \) Consumption and Phosphorylation of Isolated Pea Mitochondria**

<table>
<thead>
<tr>
<th>Basal medium plus</th>
<th>( \mu )moles O/hr</th>
<th>( \mu )moles P esterified/hr</th>
<th>P:O ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additions</td>
<td>54.6</td>
<td>90.0</td>
<td>1.65</td>
</tr>
<tr>
<td>0.08 % Pluronic F-68</td>
<td>63.6</td>
<td>96.6</td>
<td>1.52</td>
</tr>
<tr>
<td>0.08 % Pluronic F-68 and 2 ( \mu )M triolein</td>
<td>68.5</td>
<td>95.9</td>
<td>1.40</td>
</tr>
<tr>
<td>0.08 % Pluronic F-68 and 20 ( \mu )M triolein</td>
<td>63.1</td>
<td>97.2</td>
<td>1.54</td>
</tr>
</tbody>
</table>

**Table V. Effects of Uncoupling Agents and Triolein on \( O_2 \) Consumption and Fresh Weight Increases of Pea Stem Sections**

<table>
<thead>
<tr>
<th>20 ( \mu )M Triolein</th>
<th>( 5 \times 10^{-3} ) M DNP</th>
<th>( 10^{-6} ) M CCCP</th>
<th>Fr wt increase mg/5 sections per 3 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( 5 \times 10^{-3} ) M DNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>362</td>
<td>339</td>
</tr>
<tr>
<td></td>
<td>393</td>
<td>493</td>
<td>382</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>125</td>
<td>85</td>
</tr>
</tbody>
</table>

---

Copyright © 1966 American Society of Plant Biologists. All rights reserved.
so the stimulation of respiration is being studied at the same time that growth is increased. It is important to know when the triolein-induced growth occurs since some substances, especially sucrose, cobalt, and potassium, prolong the initial growth rate so that most of their effect is not found for several hours.

The maximum rate of elongation of the sections in these experiments does exceed the normal growth rate of the intact stem, at least for several hours. The lipid stimulation of growth was discovered when it was shown that sections had grown less after 24 hours than the intact stem (22). The difference in growth between the stems and sections could be reduced by adding lipids to the sections. It has now been shown that the remaining difference is due to the premature decline of the growth rate of the sections. These results suggest that we know most of the exogenous factors that increase the initial growth rate but that we know much less about keeping the sections growing.

The premature cessation of growth is not due to the accumulation of an inhibitor or depletion of an added growth factor since repeated transfer of sections to fresh media (Penny, unpublished experiments) or growing the sections in a continually changing medium (Galston, unpublished) does not increase growth. It is possible that there is a depletion of an endogenous growth factor. Adenine and some of its nucleotides can prolong the growth of sections from both Alaska peas and Arena (15, 25) but adenine has little effect on the growth of these sections (Stowe, unpublished).

The decline of the growth rate could be due to the premature onset of the maturation phase. With roots it has been possible to delay the decline of the growth rate by interfering with protein synthesis (3) but with stems these compounds cause an even faster cessation of growth (19, and unpublished experiments).

A. At one time during this study it was thought quite likely that the lipids would have to be metabolized to phospholipids before they would increase growth and respiration. This was because a large proportion of C14-methyl oleate was recovered in the phospholipid fraction, and because of the known importance of phospholipids in activating respiratory enzymes (5, 8) and in membranes. Phospholipids are not active in the bioassay, but probably do not enter the sections (Stowe and Ohrer, unpublished). Because oleic acid, which is inactive in this test, is much more readily converted into phospholipids than are active molecules it seems probable that the active lipid molecules in vivo are the same molecules that are added in the medium. It would be desirable to find an active lipid which was not metabolized by the sections or at least not converted into phospholipids. The ethyl ester of clawshoothogenic acid, the alcohols from 22 carbon acids of behenic and erucic acid, and palmitonitrile are active and are unlikely to enter phospholipids, but the radioactive forms of these molecules are not yet available to follow their metabolism. Other lines of evidence that phospholipids are not involved have been mentioned previously (22).

It has been suggested that the site of action of the lipids is on the mitochondria, because of the close similarity of the lipids that are active in this bioassay to those that reactivate isooctane extracted mitochondria (23). As yet no stimulation of the isolated pea mitochondria has been found. The reactivation by lipids of isooctane extracted mitochondria was originally interpreted as a replacement of essential lipids that had been removed by isooctane. A different interpretation is now accepted by most workers (10). Isooctane is thought to inhibit the mitochondria and the lipids displace isooctane and allow the mitochondria to function again. This theory does not assign any specific function to the lipids in the mitochondria and the analogy between the 2 systems may not be as rewarding as it once appeared to be.

When it is known what reaction is limiting respiration in the absence of triolein, then it may be possible to test the effect of triolein on this reaction in a subcellular system. The lack of stimulation of respiration by uncoupling agents helps understand the regulation of respiration in these sections and at present it seems that in this system respiration is not limited by phosphorylation or by the level of phosphate acceptors. Marre and Forti (14) report another case where a growth factor increases respiration above the maximum obtained with uncoupling agents.

The results presented thus far help to reduce the number of explanations for the apparent correlation between the lipid-induced increased respiration and the increase in the growth rate. The following 4 possibilities can be discussed: 1) The increase in growth causes the increase in respiration, possibly by an increased turnover of ATP. This has been shown to be highly unlikely since lipids will increase respiration under conditions when there are no effects on growth (18).

2) There is no causal connection between the effects of lipids on growth and respiration and the lipids may act at 2 sites. The present evidence makes it likely that only 1 site is involved since 4 different types of compounds increase both growth and respiration, in either Alaska or Progress no. 9 peas, and both are promoted by the same concentrations of triolein (18). It was possible that free fatty acids derived from the esters uncoupled respiration (21) but the lack of stimulation by uncouplers excludes this explanation. The triolein-induced respiration most likely goes through cytochrome oxidase, whose functioning is required for growth (9).

3) There is 1 site at which lipids act increasing both growth and respiration. If the lipids increased growth through a reaction that utilized ATP then respiration would also be stimulated because of a higher level of ADP or Pi. Again, the results with uncoupling agents indicate that phosphorylation is not limiting respiration in this tissue.

The interpretation that is best supported by the available evidence is: 4) Respiration or the availabil-
ity of ATP is limiting growth and that the increase in respiration induced by the lipids causes the increase in growth. The most definitive evidence comes from the lack of effect of uncoupling agents in the absence of triolein. Several interpretations have been considered (see Results) but the most probable does seem to be that phosphorylation is not limiting respiration. A recent suggestion assigns NADH a role (4).

If respiration is limiting growth, as is suggested here, then it may also be limiting other reactions. In some preliminary experiments $P^{32}$ has been fed to the sections for half an hour and then extracted by a method to inactivate phosphatases (2). No consistent effects on the uptake of $P^{32}$ has been found, but of the $P^{32}$ taken into the tissue a larger fraction is found in the organic esters and phospholipid fractions. This could be expected if triolein was increasing phosphorylation in the sections.

Acknowledgments

We are obliged to Professor G. R. Wyatt for the use of a scintillation counter and his advice. David Penny (Pennies) the New Zealand University Grants Committee for the award of a MacMillan Brown Agricultural Research Scholarship and Yale University for fellowships in the years 1962-1965.

Literature Cited


