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# STARCH PRODUCTION FROM ENDOGENOUS SUGARS IN QUIESCENT LEMON FRUIT CELLS

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**Key Word Index**—*Citrus limon*; Rutaceae; lemon fruits; starch synthesis; endogenous sugars; non-aqueous environments; liquid paraffin.

**Abstract**—Solutions of sucrose induces starch production in non-growing lemon fruit cells whereas water alone does not induce starch production. In non-aqueous environments such as olive oil and liquid paraffin, starch formation occurs in the absence of any added external sugar supply. Thus the non-aqueous media provide a physiological situation which induces the quiescent cells to utilize their endogenous sugar for starch production.

## INTRODUCTION

ALTHOUGH a mature lemon fruit is a senescing organ,[1] tissue explants from mature lemon fruits are capable of indefinite cell proliferation and growth *in vitro*.[2–4] During growth *in vitro*, citrus fruit explants synthesize a variety of materials that are normally associated with citrus fruit development under natural conditions.[4–7] However, it has been pointed out that plant cells proliferating *in vitro* bear little resemblance to the comparable cells found in the intact systems.[8] For this reason, physiological investigations have been conducted on quiescent explants from mature lemon fruits, in the absence of cell proliferation and growth.[9–11] Starch production from endogenous sugars in non-growing lemon fruit explants exposed to non-aqueous environments is reported here.

## RESULTS AND DISCUSSION

Starch-containing bodies were not evident in the freshly excised juice vesicles (histochemical control) or in the vesicles of the distilled water treatment. Starch granules were

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evident in the vesicles of the sucrose, olive oil, and liquid paraffin treatments. There was no apparent difference between the vesicles immersed in autoclaved and unsterilized liquid paraffin with respect to starch formation and there was no apparent evidence of microbial contamination of the vesicles incubated in the unsterilized liquid paraffin. Likewise there was no apparent evidence of microbial contamination of the vesicles incubated in the unsterilized olive oil.

Liquid paraffin is a highly refined petroleum product containing appreciable amounts of naphthenic rings with paraffinic side chains and is in general physiologically harmless and chemically inert.[12–15] Olive oil, however, is not physiologically inert and in addition, exerts an apparent solvent action upon sucrose (unpublished observation). This apparent solvent action of olive oil on sucrose suggested the possibility that olive oil contained free sugar(s) which would induce starch formation in the vesicle cells. An analysis of aqueous extracts of 200 $cm^3$ samples of olive oil revealed trace amounts of free D-galactose, free D-glucose, and 1·62–1·75 $\mu g/cm^3$ of cysteine–sulphuric acid positive material almost half of which was also soluble in chloroform. The amounts of free galactose and free glucose were too small to measure quantitatively and it is doubtful, therefore, whether such small amounts of free sugar in the olive oil were responsible for the induction of starch formation in the vesicle cells. Acid hydrolysis of olive oil for 16 hr yielded 2·3 $\mu g/cm^3$ oil of D-galactose, 1·91 $\mu g/cm^3$ oil of D-glucose, and a syrupy mass which indicated that glycolipids were being hydrolyzed. Whether the water-soluble cysteine–sulphuric acid positive material from the olive oil or the glycolipids were involved in the activation of starch production in the juice vesicles is not known. However, the results obtained with liquid paraffin concerning the induction of starch formation in the vesicle cells in the absence of any exogenous sugar supply indicate that olive oil induces starch production in the cells in a manner similar to that of liquid paraffin, namely by a physical rather than a chemical effect.

Juice vesicles of lemon fruits as well as other citrus fruits are known to contain sugars.[16,17] What is of interest here is that the quiescent explants are induced into utilizing their endogenous sugar for starch production when in a non-aqueous environment whereas the quiescent explants are not induced into utilizing their endogenous sugar for starch production when placed on distilled water alone. Thus starch formation occurs in the vesicles when placed in an aqueous environment containing an exogenous sugar supply whereas starch production occurs in this tissue system in the absence of any external sugar supply when placed in a non-aqueous environment. The reasons for such differences in behaviour of the juice vesicles regarding the induction of starch production in aqueous and non-aqueous environments cannot be explained at the present time.

## EXPERIMENTAL

Firm mature yellow lemon fruits (*Citrus limon* L.) with green buttons were surface-sterilized with a calcium or sodium hypochlorite solution and entire juice vesicles (sac plus stalk) were; (i) floated on the surface of glass-distilled water, (ii) floated on the surface of a 4% sucrose solution, (iii) completely immersed

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in B.P. Standard olive oil[18] or Fisons Laboratory Reagent olive oil (Fisons Scientific Apparatus Ltd., Loughborough, England) 3–4 mm deep, and (iv) completely immersed in BDH Laboratory Reagent Light Grade liquid paraffin (BDH Chemicals Ltd., Poole, England) 3–4 mm deep in Pyrex Petri dishes immediately upon removal from the fruit. The Petri dishes in all four treatments were sealed with Parafilm and placed in the dark for 72 hr at 25–27°. Sucrose was autoclaved in the crystalline state and autoclaved distilled water was added to the sucrose crystals after both had cooled to room temperature.[6] Unsterilized and autoclaved liquid paraffin were used whereas the olive oil was not sterilized or heated in any way. The Petri dishes used for all four treatments were sterilized by autoclaving.

After 72 hr, the juice vesicles from all four treatments were placed in a dilute KI solution for 48 hr or longer and then examined for starch granules in an intact and unsectioned condition.[9] (*Note.* Iodine crystals are completely soluble in olive oil and in liquid paraffin. Consequently, any oily film that may be present on the surface of the vesicles will not prevent the entry of iodine into the cells.) Juice vesicles placed in the KI solution immediately upon removal from the fruit (freshly excised vesicles) served as the histochemical control.

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