# CHEMICAL CHANGES DURING GROWTH AND STORAGE OF THE AVOCADO FRUIT

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[*Manuscript received May* 13, 1959]

*Summary*

Changes in the petrol-soluble, 80 per cent. ethanol-soluble, and insoluble fractions of the mesocarp of the avocado fruit were followed during 6 months development on the tree and during storage at 20°C of the fully grown fruit. Changes in triglyceride fatty acids, lipid phosphorus, insoluble phosphorus, sugars, protein, and soluble nitrogen were also studied.

During development there was a large increase in the triglyceride oil content with a concomitant fall in water content. Monoene acids were principally synthesized, the saturated and polyene acids being laid down at an early stage in the development of the fruit. A considerable proportion of polar homolipids was shown to be present in the fruit.

The $C_7$ sugars may play an important role in the metabolism of the fruit and lipid material probably contributes to respiration. Phospholipid content was high in the young fruit but fell in the first month to a steady value. Synthesis of protein occurred during storage and the changes taking place during storage varied according to the stage of development of the fruit.

## I. INTRODUCTION

Studies on the avocado fruit were undertaken primarily to elucidate the pattern of fatty acid synthesis during growth and storage, and to relate this to other chemical changes in the mesocarp tissue. Early studies of the chemical constitution of the mesocarp of the fruit of *Persea gratissima* (*P. americana*) were reviewed by Wardlaw (1937). Interesting features of the chemical composition are the very high fat content (up to 30 per cent. of the fresh tissue) and the presence of a heptitol, perseitol (Maquenne 1890), and an aldoheptose, *manno*heptulose (La Forge 1916). Investigation of the composition of the triglyceride oil (Asenjo and Goyco 1942; Alvarez *et al.* 1949) has revealed the presence of myristic, palmitic, stearic, arachidic, palmitoleic, oleic, docosenoic, linoleic, and linolenic acids. The work described here shows that the diene and triene acids are laid down principally in the very young fruit, and subsequent fatty acid synthesis is mainly of monoene acids. The fruit contains a considerable amount of homolipids more polar than the triglycerides and the data suggested that the $C_7$ sugars may play an important part in the metabolism.

## II. METHODS

### (*a*) *Sampling*

Fruit of the Fuerte variety was obtained from a farm at Redland Bay, S. Qld., at the beginning of each month from January to June 1957. It was flown to Sydney on the day of picking, and analysis commenced the next day. The fruit

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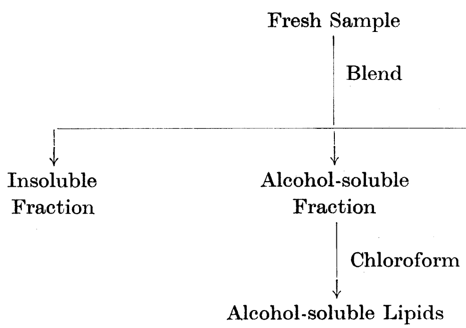
was picked from four trees, the fourth unfortunately yielding no more fruit after the April picking. Details of these pickings are set out in Table 1. The fruit was fully grown by April though May is considered the month of commercial maturity. The fruit in May was slightly larger than in June, probably due to selective picking of the larger fruit. In January, February, and March the fruits were weighed on arrival and divided into two samples, A and B, each sample containing the same number of fruits from each tree and with a similar range of individual fruit weights. In April, May, and June, the batch was divided into four samples A, B, C, and D on the same basis. A and B were analysed immediately, while C and D were analysed after storage for a period at 20°C, during which respiration was measured and the fruit became ripe (i.e. soft and edible).

For analysis, a longitudinal segment was cut from each fruit and peeled. A thin longitudinal slice of constant thickness was cut from each segment, halved, and alternate distal and stem end pieces combined in the sample, which usually weighed 25–30 g. Comparable duplicate samples were used for moisture determinations.

### (b) *Fractionation*

The fresh sample was blended with ethanol (sufficient to give a final concentration of 80 per cent. aqueous ethanol) and light petroleum (b.p. 60–70°C) at 1°C. After blending, the insoluble residue was filtered off, washed thoroughly with 80 per cent. aqueous ethanol and light petroleum, dried, and weighed. This was a light-coloured friable powder. The two phases of the filtrate were separated. The petrol-soluble material was weighed after evaporation of the petrol, and the ethanol-soluble fraction was diluted to 250 ml. An aliquot of this alcoholic solution was evaporated to dryness and weighed to determine alcohol-soluble solids. The dry residue was extracted with chloroform and the chloroform extract was weighed after evaporation of the solvent. The fractionation procedure is summarized in the following diagram:




### (c) *Analysis*

Nitrogen and phosphorus were determined on the insoluble fraction and reducing sugars and nitrogen on the alcohol-soluble fraction. The petrol-soluble fraction was saponified and acid value, iodine value, and ultraviolet absorption after alkaline isomerization determined on the isolated acids. Phosphorus content and ultraviolet absorption after alkaline isomerization were determined on the alcohol-soluble lipids. The following analytical procedures were used: nitrogen by the microKjeldahl procedure of McKenzie and Wallace (1954); lipid phosphorus by the method of Allen (1940) as modified by Rhodes (1955); insoluble phosphorus by the method of Ging (1956); reducing sugars by the ferricyanide method of Ting (1956); acid value by the official method of the American Oil Chemists' Society (1946*a*); iodine value by the official method of the American Oil Chemists' Society (1946*b*); alkaline isomerization with $1 \cdot 0$M potassium *tert.*-butoxide for 2 hr at 100°C (Davenport, Birch, and Ryan 1956), the percentages of the various fatty acids being calculated using the following equations:

$$\text{Percentage triene acid} = 1 \cdot 314k_{268}.$$
$$\text{Percentage diene acid} = 1 \cdot 013k_{233} - 0 \cdot 734k_{268}.$$

Table 1

DETAILS OF SAMPLING OF AVOCADO FRUITS

| Date of Picking | No. of Trees | No. of Fruit from Each Tree | Average Weight (g) | Range in Weight (g) |
|---|---|---|---|---|
| 8. i.57 | 4 | 8 | 99 | 58–147 |
| 8. ii.57 | 4 | 8 | 195 | 144–264 |
| 6.iii.57 | 4 | 8 | 288 | 181–379 |
| 3.iv.57 | 4 | 16 | 296 | 213–444 |
| 1. v.57 | 3 | 8 | 309 | 226–422 |
| 3.vi.57 | 3 | 16 | 299 | 169–417 |

The monoene acid and saturated acid content was calculated in the usual way from the iodine value and percentages of diene and triene acids. The residue from the alcohol-soluble fraction after extraction with chloroform was de-ionized with a mixture of "Amberlite" resins (IR–4B.OH and IR–120.H) and the residual sugars were chromatographed on paper with ethyl acetate–*n*-propanol–water (7 : 2: 1 by volume) as solvent. The papers were sprayed with alkaline silver nitrate (Trevelyan, Procter, and Harrison 1950) and with the orcinol–trichloroacetic acid–acetic acid reagent of Klevstrand and Nordal (1950). The latter gave a blue colour with *manno*heptulose, a pale green with fructose, and did not react with the other sugars present.

## III. Results

### (*a*) *Segregation in the Fractionation Procedure*

It could be assumed on the basis of known solubilities that the petrol-soluble fraction would contain the triglycerides and unsaponifiable matter, the alcohol-soluble fraction reducing sugars, amino acids, and phospholipids, and the insoluble fraction polysaccharides and proteins. None of the petrol-soluble fractions contained phosphorus, hence no phospholipid went into the petrol phase. A surprising feature was the high proportion (30 per cent. or greater) of the alcohol-soluble fraction which was soluble in chloroform. Phosphorus analyses indicated that the alcohol-soluble lipids contained approximately 10 per cent. phospholipid and, consistent with this, approximately 10 per cent. was insoluble in acetone. A preliminary examination of the acetone-soluble alcohol-soluble lipids by absorption

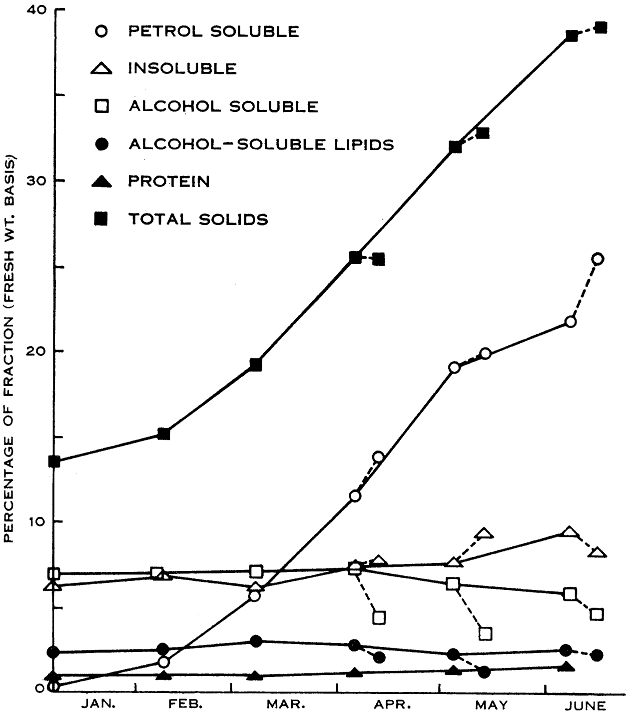



Fig. 1.—Percentage changes, on a fresh weight basis, of the various fractions of the mesocarp during development (solid lines) and storage (broken lines). The standard errors of the means, with nine degrees of freedom, were as follows: petrol-soluble fraction, 0·48; insoluble fraction, 0·31; alcohol-soluble fraction, 0·25; alcohol-soluble lipids, 0·11; protein, 0·04; total solids, 0·49.

chromatography revealed that it consisted of a complex mixture of lipids containing only carbon, hydrogen, and oxygen. Further chemical work on the characterization of these lipids is in progress and will be reported separately. They appear to be lipids of a type not previously described.

Also, the triglycerides were confined wholly to the petrol phase, none or very little appearing in the alcohol-soluble lipids fraction. Chromatographic examination of the combined unsaponifiable matter from the petrol-soluble fractions revealed that it consisted principally of hydrocarbon and sterol fractions and contained negligible amounts of the substances present in the alcohol-soluble lipids fraction.

Hence it appears that the fractionation achieved good segregation of the various lipid types. A considerable amount of phosphorus was found in the insoluble fraction and initially it was thought that this might be due to the phospholipid associated with the protein. Hence part of the insoluble fraction of sample A (June picking) was refluxed with an ethanol–diethyl ether (3 : 1 v/v) mixture, and filtered. The filtrate was taken to dryness, yielding 5·2 per cent. of lipid which contained no phosphorus and which was almost completely soluble in light petroleum. This appears to be lipid more firmly bound to protein or polysaccharide or both than that in either the petrol-soluble or the alcohol-soluble lipids fractions. It was found that all the insoluble phosphorus could be extracted in the

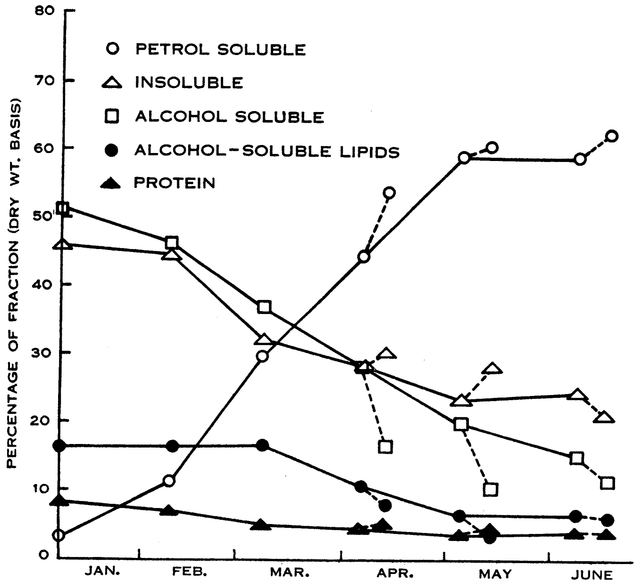



Fig. 2.—Percentage changes, on a dry weight basis, of the various fractions of the mesocarp during development (solid lines) and storage (broken lines).

cold with 1·5N perchloric acid, and that the extract showed a fairly broad absorption maximum at 256 m$\mu$, indicating the presence of nucleotides. However, the insoluble residue of sample A (June picking) contained 6·8 mg-atoms of phosphorus and only 0·025 m-moles of nucleotide (assuming an extinction coefficient ($\epsilon$) of 10,000). Hence most of the insoluble phosphorus is present in other forms.

### (*b*) *Changes during Development of the Fruit on the Tree*

Percentage changes in the insoluble, alcohol-soluble, petrol-soluble, and alcohol-soluble lipids fractions have been plotted on a fresh weight basis in Figure 1, and on a dry weight basis in Figure 2. Percentage protein has been calculated by multiplying the percentage insoluble nitrogen by 6·25. The analytical values for nitrogen, phosphorus, and reducing sugars of the various fractions are plotted on a fresh weight basis in Figure 3 and on a dry weight basis in Figure 4. Changes in the percentages of the various types of fatty acids in the petrol-soluble fraction are plotted on a dry weight basis in Figure 5, and the results of the alkaline isomerization of the alcohol-soluble lipids fractions listed in Table 2. Each point on the graphs is the average of values for the duplicate fruit samples.

In Figure 1 and 2 it may be seen that marked synthesis of triglyceride oil took place mainly over a 3-month period (from February to April). In May the apparent increase in oil on a fresh weight basis was merely due to a loss of water from the fruit. Reference to Figure 5 indicates that it was principally monoene acids which were being synthesized, saturated and diene acids increasing only slightly, and triene acids remaining virtually constant.

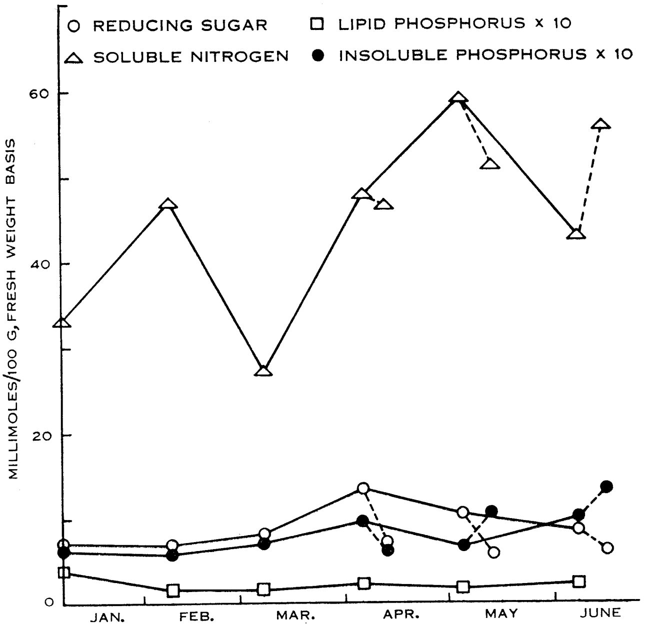



Fig. 3.—Changes on a fresh weight basis of various chemical entities in the mesocarp during development (solid lines) and storage (broken lines). The standard errors, with nine degrees of freedom, were as follows: reducing sugar, 0·84; soluble nitrogen (alcohol-soluble fraction), 2·3; lipid phosphorus, 0·03; insoluble phosphorus, 0·05.

The concentrations of insoluble, alcohol-soluble, alcohol-soluble lipids fractions and protein on a fresh weight basis did not change markedly, their apparent decrease on a dry weight basis being due to a proportional increase in oil content. In May, however, there was a definite fall in the alcohol-soluble fraction which was due mainly to a fall in reducing sugar concentration and some loss of soluble nitrogenous compounds. The phospholipid content fell sharply in January and thereafter remained fairly constant. The isomerization analysis of alcohol-soluble lipids showed a sharp rise in conjugatable diene and a fall in conjugatable triene during January and both then remained fairly constant.

From January to May there was a parallel between changes in reducing sugar and in insoluble phosphorus. In May, however, the reducing sugars continued to fall, whereas the insoluble phosphorus increased. Paper chromatography of the sugars revealed the presence in each monthly sample of sucrose, perseitol, *manno*-heptulose, and an unknown sugar, possibly a disaccharide, with an $R_F$ near maltose (hereafter referred to as the unknown disaccharide). With the solvent system used, *manno*heptulose and glucose were not separated, but a comparison of the intensity of the reactions with alkaline silver nitrate and orcinol–trichloroacetic acid indicated

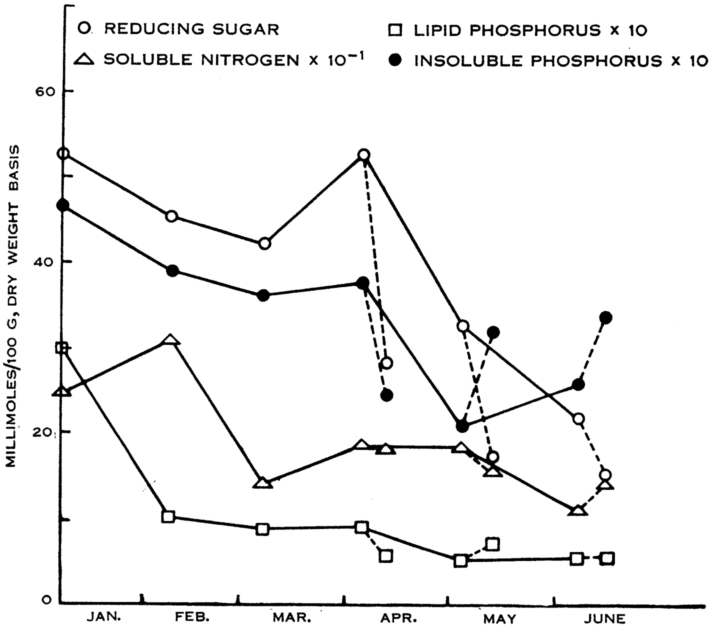



Fig. 4.—Changes on a dry weight basis of various chemical entities in the mesocarp during development (solid lines) and storage (broken lines).

that glucose was present. Paper chromatography did not reveal any marked variation in the proportion of the various sugars throughout the period studied except in May, when the perseitol concentration was higher than in the other months.

### (c) *Changes during Storage*

The respiration of the three stored samples was measured. The April sample did not pass through a well-defined climacteric and the fruit had only partly softened when it was analysed. In May and June the respiration exhibited the usual well-defined climacteric and the analysed samples were typically soft and edible. In Figures 1–5, the changes during storage are represented by the broken lines.

It is apparent from the figures that the changes during storage were dependent upon the stage of development of the fruit on the tree. There was only a slight amount of fat synthesized during storage, and marked falls in the alcohol-soluble fraction, due principally to a loss of sugar and, to a lesser extent, of lipids. Paper chromatography of the sugars showed that in April the perseitol almost disappeared, the *manno*heptulose decreased slightly, fructose and sucrose increased,

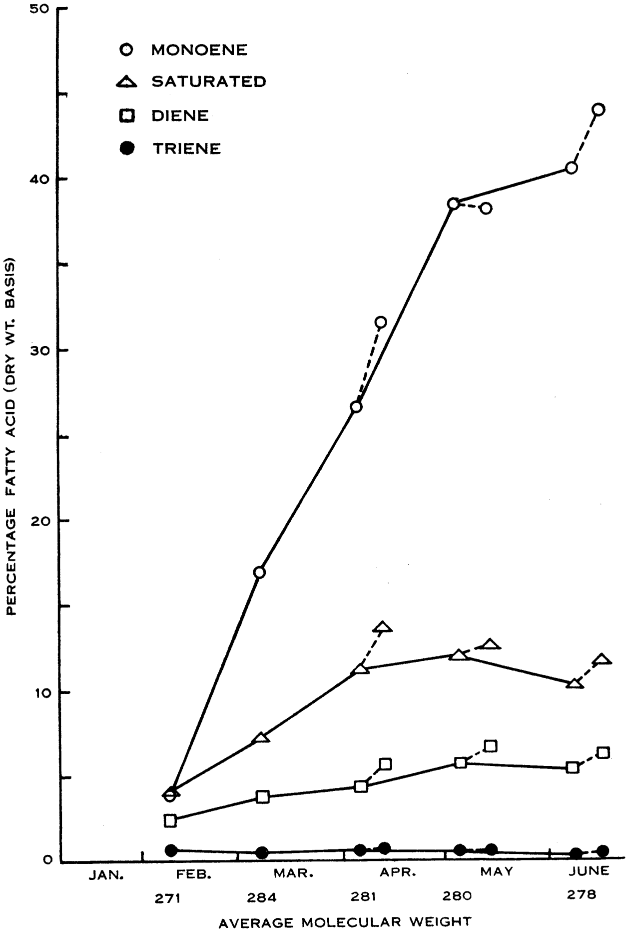



Fig. 5.—Percentage changes, on a dry weight basis, of the various classes of petrol-soluble fatty acids during development (solid lines) and storage (broken lines). The standard errors, with seven degrees of freedom, were as follows: saturated, 0·65; monoene, 1·56; diene, 0·14; triene, 0·13.

and the unknown disaccharide increased. In May the perseitol and *manno*heptulose almost disappeared, the fructose and the unknown disaccharide increased markedly, and the sucrose did not change appreciably. In June both perseitol and *manno*heptulose practically disappeared, fructose increased markedly, while sucrose and the unknown disaccharide both increased. In April and May there was a slight synthesis of protein and a somewhat greater increase of insoluble material. Synthesis of protein during storage has also been noted, and correlated with high energy phosphate and the climacteric rise of the fruit by Rowan, Pratt, and Robertson (1958). In June, however, the protein did not change and the insoluble fraction decreased markedly. The insoluble phosphorus increased in April but decreased in May and June. There were no striking changes in lipid phosphorus or soluble nitrogen except for a marked increase of the latter in June.

TABLE 2

ALKALI ISOMERIZATION OF ALCOHOL-SOLUBLE LIPIDS*

Results are expressed as extinctions ($E^{1\%}_{1\,cm}$) at the wavelengths for conjugated diene (233 m$\mu$) and conjugated triene (268 m$\mu$) developed after isomerization

| Wavelength | Jan. | Feb. | Mar. | April | | May | | June | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Before Storage | After Storage | Before Storage | After Storage | Before Storage | After Storage |
| 233 m$\mu$ | 131 | 229 | 230 | 237 | 236 | 237 | 237 | 237 | 260 |
| 268 m$\mu$ | 86 | 46 | 31 | 31 | 33 | 43 | 51 | 41 | 34 |

* Preliminary work on the acetone-soluble portion of the alcohol-soluble lipids fraction indicates that this conjugation does not develop necessarily from unsaturated fatty acids, although some may arise from unsaturated fatty acids in the phospholipids.

## IV. Discussion

A microscopic examination of the avocado mesocarp shows that the fat droplets accumulate in the vacuole of the cells. In the ripe fruit the vacuole presents a striking appearance, being packed with oil droplets. The fall in the water content of the fruit during development may be due to a displacement of water from the vacuoles by the accumulating oil droplets. During the phase of rapid fat synthesis, monoene acids were almost exclusively synthesized. The Fuerte avocado oil is unusual in that it contains about 6 per cent. of palmitoleic acid and about 3 per cent. of docosenoic acid, as well as about 55 per cent. of oleic acid (Alvarez *et al.* 1949). The average molecular weight of the fatty acids was constant throughout, and possibly all three monoene acids were being synthesized. Stumpf and Barber (1957) have demonstrated that mitochondria isolated from avocados incorporate labelled acetate into oleic and palmitic acids under anaerobic conditions, most activity appearing in the former. This result is consistent with the predominant synthesis of monoene acids by the whole fruit.

The early rise in conjugatable diene and fall in conjugatable triene in the alcohol-soluble lipids appeared to be associated with a fall in the phospholipid content of that fraction, and lend support to the concept of an early establishment of the pattern of polyunsaturated acids in the fruit, which then remains fairly constant throughout subsequent development and storage. During storage in May and June there were small but significant changes in the composition of the oil. The monoene acid content fell while that of the saturated, diene, and triene acids tended to rise, suggesting that the fatty acids are involved, to some extent, in metabolic changes during storage. The alcohol-soluble lipids, as well as the reducing sugars, appeared to contribute to the respiration during storage, and this may account for the low R.Q. observed by Biale (1946).

During storage the $C_7$ sugars almost disappeared, leaving the more usual pattern of $C_6$ sugars. A simple explanation is that the $C_7$ sugars were not synthesized in the fruit, but translocated to it from other parts of the tree; hence, when the fruit was picked, they were metabolized with no replacement. Nordal and Benson (1954) have isolated *manno*heptulose and its monophosphate from avocado leaves. They were unable to isolate it from several varieties of the fruit, and this may have been due to the fact that ripe fruit was used. They concluded that heptulose metabolism in the avocado was sluggish and did not play an important role in the metabolism of the plant, but the present results indicate that it is an active substrate for respiration in the fruit.

## V. Acknowledgments

The authors are indebted to Dr. A. A. Benson for a gift of authentic samples of perseitol and *manno*heptulose, to Dr. J. F. Turner who performed the paper chromatography of the sugars, and to Drs. R. N. Robertson, F. E. Huelin, J. F. Turner, D. H. Turner, and K. S. Rowan for helpful discussion. We are also indebted to Mr. H. W. L. White, Orchardist, Redland Bay, Qld. for his helpful cooperation.

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