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SHORT COMMUNICATION

CHANGES IN TOTAL RIBONUCLEIC ACID DURING THE CLIMACTERIC PHASE IN YELLOW TRANSPARENT APPLES*

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Abstract—Total ribonucleic acid (RNA) was determined in cortical tissue of ripening Yellow Transparent apples. The rate of respiration of composite fruit samples was also determined. Total RNA increased markedly as the respiration rate increased. Ratios of individual nucleotides in the total RNA fraction did not change appreciably. The relationship of this quantitative change in total RNA to protein synthesis during the climacteric stage is discussed.

INTRODUCTION

THE CLIMACTERIC rise in respiration is exhibited by many fruits and has been investigated by physiologists and biochemists since it was reported by Kidd and West[1] in 1925. This phenomenon is recognized as being of both practical and theoretical interest because of its relation to senescence of the organ. The attempts to explain the climacteric can be broadly categorized into two approaches. The first has been to relate a senescent loss of structural integrity to the respiratory rise due to loss of cellular membrane control of substrate-enzyme associations. Blackman and Parija[2] and more recently Bain and Mercer[3] and Sacher[4] have defended this alternative. The second line of investigation has been more biochemical in nature and has included work in respiratory control,[5] on the activity of various catabolic enzyme systems,[6] and on the occurrence of biosynthetic activity such as protein and ethylene synthesis. All of these lines of investigation have been combined in various ways. Pearson and Robertson,[7] for instance, pointed to the relationship between biosynthesis and the stimulation of terminal oxidation and Hulme *et al.*[8] have related enzyme protein synthesis and mitochondrial activity during the climacteric phase.

Broadly speaking, the proponents of the first approach view the climacteric as a senescent phenomenon and would argue that prior to or at the time of the climacteric rise, cellular deterioration is active. Conversely, the latter approach generally assumes that during the

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[1] F. KIDD and C. WEST, *Gt. Brit. Dep. Sci. Ind. Res. Food Invest. Bd. Rep. for 1924*, p. 27 (1925).
[2] F. F. BLACKMAN and P. PARIJA, *Proc. Roy. Soc.* (*London*) **B103**, 412 (1928).
[3] J. M. BAIN and F. V. MERCER, *Australian J. Biol. Sci.* **17**, 78 (1964).
[4] J. A. SACHER, *Plant Physiol.* **41**, 701 (1966).
[5] C. LANCE, G. E. HOBSON, ROY E. YOUNG and J. B. BIALE, *Plant Physiol.* **40**, 1116 (1966).
[6] J. BARKER and T. SOLOMOS, *Nature* **196**, 189 (1962).
[7] J. A. PEARSON and R. N. ROBERTSON, *Australian J. Biol. Sci.* **7**, 1 (1954).
[8] A. C. HULME, J. D. JONES and L. S. C. WOOLTORTON, *Proc. Roy. Soc.* (*London*) **B158**, 514 (1963).

climacteric phase anabolic activity occurs which is important in the subsequent senescence of the organ.

The presence or absence of protein synthesis during the climacteric phase has been investigated repeatedly in an attempt to resolve this question. The majority of workers in this area have found that at some time during the climacteric rise there is increased protein synthesis as measured either by total protein N or by rate of amino acid incorporation.[9-11] This opinion, however, has not been unanimous.[4]

The work reported in this paper was designed to shed light on this question by investigating the status of RNA in climacteric apples. It was reasoned that an increase in protein synthesis would require a concomitant increase in total RNA.

RESULTS

Figure 1 shows the relationship between total RNA content and the respiratory climacteric in Yellow Transparent apples. Beginning with the harvest of 7/18 (70 days after full bloom) the fruit was capable of exhibiting a climacteric rise in respiration in the time allotted. As the respiratory rate increased the level of total RNA increased concomitantly. When

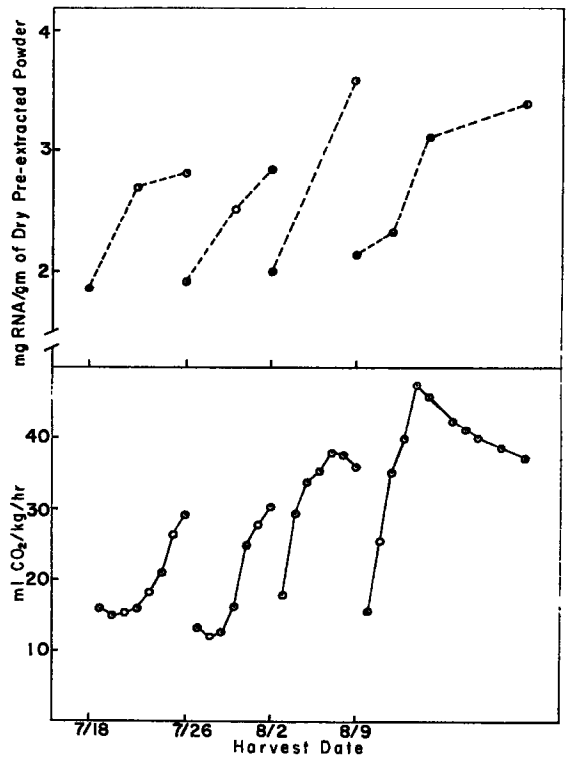

Fig. 1. Changes in RNA related to the respiratory climacteric in Yellow Transparent apples.

[9] R. S. Rowan, H. K. Pratt and R. N. Robertson, *Australian J. Biol. Sci.* **11**, 329 (1958).
[10] A. Richmond and J. B. Biale, *Plant Physiol.* **41**, 1247 (1966).
[11] A. C. Hulme, *J. Exptl Botany* **5**, 159 (1954).

assayed on the day of harvest, neither the RNA content nor the level of respiratory activity changed markedly from day one of the previous harvest series during the period shown in Fig. 1. This would be the best estimate of physiological condition of fruit on the tree and would indicate that the changes evident in fruit ripening on the tree are not as pronounced as those for detached fruit ripened in the laboratory. Inasmuch as a series of matched samples were assayed from each harvest and growth was not a consideration, the RNA data expressed on a residual dry weight basis indicate a *de novo* synthesis in the detached fruit.

The increase in RNA was not accompanied by any marked shift in the molar ratios of the four nucleotides isolated (Table 1).

TABLE 1. MOLAR PER CENT OF THE FOUR RIBONUCLEOTIDES FROM RIPENING YELLOW TRANSPARENT APPLES

Harvest date	Days of ripening	% of Each Nucleotide			
		Cytidylic acid	Adenylic acid	Uridylic acid	Guanylic acid
7/19	0	21·6	26·8	21·0	30·6
7/19	4	20·6	28·9	20·1	30·3
7/19	7	20·3	27·6	21·8	30·3
7/26	0	21·3	28·6	19·0	30·9
7/26	4	21·1	29·5	18·8	30·6
7/26	7	22·7	25·0	22·1	30·2
8/2	0	23·6	26·1	22·4	27·8
8/2	6	25·1	26·6	20·4	27·9
8/9	0	24·9	26·5	18·5	30·1
8/9	3	23·6	26·6	20·1	29·7
8/9	7	23·4	25·9	21·0	29·7
8/9	21	22·0	26·8	22·1	29·1
	$\bar{x}$	22·5	27·1	20·6	29·8

DISCUSSION

The relationship indicated in Fig. 1 is in accord with the idea that protein synthesis is active during the climacteric phase. Apple fruit cortex is very low in RNA and it was not possible to isolate the various species of RNA from one another. Nonetheless, due to the magnitude of the increase noted, it is considered likely that ribosomal RNA contributes largely to this increase. The appearance of specific proteins at this time would require the participation of messenger RNA but would not lead to an increase in total RNA of the magnitude recorded. Richmond and Biale,[12] in a recent report fractionated RNA components from avocado fruit and found 70–90 per cent of the total as ribosomal RNA. It has long been recognized that the intensity of protein synthesis is related to the amount of ribosomal RNA present.[13]

It is interesting to apply to the interpretation of these data the reasoning which Laties[14] suggested to explain the increased respiration rate of cut potato slices. He has shown that this respiratory rise is accompanied by increased RNA and protein synthesis. He then postulates that ribosome formation increases and that this synthetic activity involves both the new RNA and protein.

[12] A. RICHMOND and J. B. BIALE, *Plant Physiol. Suppl.* **41**, lxiii (1966).
[13] H. CHANTREEN, *The Biosynthesis of Proteins*. Pergamon Press, Oxford (1961).
[14] C. LATIES, *Plant Physiol.* **40**, 1237 (1965).

EXPERIMENTAL

Yellow Transparent apples were harvested weekly starting early in their developmental period and measured daily for respiration rate at 23° using the constant flow apparatus of Claypool and Keefer[15] and a Model 29 Fisher-Hamilton gas partitioner to measure the CO_2 evolved from the respiration chambers. Two chambers, each containing ten fruits were used. When the respiratory data indicated that the fruits were approaching the maturity required to ripen readily under laboratory conditions, ten matched fruit samples were subjected to the same conditions as the fruit in the respiration chambers. On the day of harvest and on later occasions as they ripened, ten duplicate fruit samples were assayed for total RNA. The last assay of each series was of the fruit in the respiration chambers.

The tissue extraction began in the enzyme laboratory at 1°. Only cortical tissue was used and during the peeling and coring operation the tissue was kept in a 1% ascorbic acid solution to prevent browning. For each assay 150 g of prepared tissue was used. The original blending was in 500 ml of 95% ethanol containing 1% polyvinyl pyrrolidone and 1% ascorbic acid. After centrifugation the supernatant was discarded and the tissue pad was extracted successively by blending and centrifuging with each of the reagents listed below, discarding all supernatants: (1) 150 ml of 95% ethanol; (2) 150 ml of 70% ethanol plus 0·1% perchloric acid; and (3) 100 ml of 95% ethanol. The tissue was then blended with 150 ml of 70% acetone and filtered over Whatman No. 42 filter paper in a Buchner funnel. The pad was boiled with ethanol/ethyl ether (3:1 vol:vol) for 3 min. and filtered. This operation was repeated. The procedure of Diener and Lasheen[16] for extraction of acid soluble compounds was used and the dried pre-extracted powder was carefully weighed. Hydrolysis of RNA and isolation of the ribonucleotides from DNA was also by the method of Diener and Lasheen.[16] The procedure of Fritz and Rottger[17] was employed for the separation of ribonucleotides by Dowex-1 ion exchange column chromatography.

[15] L. L. CLAYPOOL and R. M. KEEFER, *Proc. Am. Soc. Hort. Sci.* **40**, 177 (1942).
[16] T. O. DIENER and A. M. LASHEEN, *Proc. Am. Soc. Hort. Sci.* **75**, 195 (1960).
[17] H. FRITZ and B. ROTTGER, *Z. Naturforsch.* **18**, 124 (1963).