Permeability Characteristics and Amino Acid Incorporation during Senescence (Ripening) of Banana Tissue[1]

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Received October 25, 1965.

Summary. Changes in free space of banana tissue during ripening were measured using radioisotopes. Free space increased significantly about 44 hours before the onset of, and rose exponentially during the respiratory climacteric. The increase in free space indicates a progressive increase in the proportion of cells which becomes completely permeable to solutes in the ambient solution by simple diffusion. At the respiratory peak the tissue was essentially 100 % free space to mannitol, sucrose, fructose and chloride.

The capacity for active uptake of solutes declined about one day before the onset of the respiratory rise and fell to a very low level by the respiratory peak.

There was no change in the level of protein or amino acids during ripening. Assays of tissue sections before and after washing indicated an increased rate of leakage of amino acids during ripening.

Studies of incorporation of 3 concentrations of ^{14}C-labeled leucine and phenylalanine indicated marked changes in the size and specific activity of the amino acid pool at the site of protein synthesis just prior to and during the climacteric rise, due to a diffusive mixing of the labeled substrate with the previously sequestered endogenous, unlabeled pool of substrate. The use of a high concentration of exogenous substrate (above saturation for uptake) resulted in an apparent constant specific activity of the metabolic pool through the ripening period. Data from these studies indicated a decline in amino acid incorporation during the climacteric.

It was concluded that the initiation of permeability changes marks the onset of senescence in banana. The causative relations between alterations in permeability, the respiratory rise and other chemical changes attending fruit ripening are discussed.

Permeability changes attend senescence of bean (*Phaseolus vulgaris*, L.) endocarp sections, leaf sections of *Rhoeo discolor*, as well as petiolar abcission zones of *Coleus* sp., *Fuschia* sp. and *Cestrum nocturnum* (12, 21, 22). For these tissues auxin maintained membrane integrity.

Over 35 years ago investigators (7, 14) of the marked respiratory rise during ripening of certain fruits alluded to permeability changes as being causative. More recently it was demonstrated for banana and avocado (23) that an increase in leakage of solutes precedes or attends the onset of the climacteric, followed by an acceleration of the rate of leakage during the rise in respiration. This was attributed to changes in permeability. It was postulated that loss of permeability barriers could lead to changes in protoplasmic compartmentalization and alter relations between enzymes and substrates, and thereby affect the rate of respiration and other metabolic changes associated with the climacteric. A similar suggestion was made on the basis of the observation of an inverse correlation between respiration and electrical impedance of avocado tissue during ripening (3). Also for avocado a 3-fold increase in free space was observed during ripening (4), although the method used was not precise enough to ascertain if the increase in free space preceded the respiratory rise.

Other investigators (9) concluded that membrane permeability remains constant during banana ripening and the increased leakage is simply (from Fick's law) a manifestation of the increase in endogenous levels of sugars. Subsequently it was reported that for banana tissue there occurred an increase in efflux of salts from tissue sections during ripening (2).

Because of the significance of changes in membrane permeability to cellular compartmentalization and thereby to relations between enzymes and substrates in general, more detailed quantitative studies were conducted to examine cellular phenomena which could be affected by permeability characteristics. This paper reports several types of evidence for banana tissue which demonstrate that preceding the climacteric there occurs an increase in free space, a dimin-

[1] This investigation was supported by Grant GB-287 from the National Science Foundation.

ution in active uptake of ions and organic solutes and an increase in the rate of leakage of amino acids, and that all these changes are vastly augmented during the climacteric rise. Also discussed are the results of studies of amino acid incorporation which indicate marked changes in the size of the amino acid pool at the site of protein synthesis just prior to the onset of and during the climacteric rise. From these data and protein assays an assessment of the rate of protein turnover is made.

Materials and Methods

Hands of green bananas (*Musa sapientum* L., var. Gros Michel) were obtained from McCann Produce Company, Los Angeles, and maintained at 22° and high relative humidity. Respiratory measurements of whole bananas were based on evolution of CO_2, measured with a Beckman Model 15A infrared CO_2 analyzer and recorder as previously described (23). The banana used for daily experiments was removed from the respiration chamber after an assay of CO_2 evolution for a period of 4 to 20 hours.

There was no significant difference in the basal (preclimacteric) respiration rate among different bananas from the same hand, nor in the rate of increase in CO_2 evolution during the respiratory rise. Randomized duplicate or triplicate batches of tissue were prepared daily for use in the procedures described below. There was variation among different hands of bananas in the number of days from the start of the experiments to the onset of the climacteric (e.g., from 2–7 days), probably due to factors such as time of picking and conditions and duration of transit.

Protein Assays. Samples of 2 g each were weighed daily and then dried in a desiccator over concentrated H_2SO_4 under vacuum for determination of dry weights. The dry weight/fresh weight ratio of pulp tissue from 17 bananas used over a ripening period of 14 days averaged 0.2849 ± 0.0024 (SE). The dried tissue was powdered and 50 mg portions in duplicate were washed 3 times with cold 5 % trichloroacetic acid in a Potter-Elvejhem homogenizer, followed by centrifugation at 1° to precipitate protein. The final precipitate was digested and assayed for Kjeldahl nitrogen as described by Lang (15). The value in mg was multiplied by the factor 6.25 to give an approximation of mg protein.

Measurement of Free Space. Determination of the percentage of the tissue volume that is free space (cf. 11 for a discussion of free space) was by measurement of the uptake of radioactive solutes into tissue discs. Discs (1 × 5 mm) were prepared from banana tissue with a hand microtome and a No. 2 cork borer, washed and randomized for 5 minutes in running tap water and then blotted gently on Whatman No. 1 paper. Assays were made with duplicate batches of 12 discs (256 mg fr wt) incubated for 20 minutes at 29° in 0.4 ml solutions, buffered to pH 7.0 with 0.03 M phosphate buffer and containing 0.1 M mannitol-1-^{14}C, 0.03 M sucrose-U-^{14}C, 0.06 M fructose-U-^{14}C or 0.07 M potassium chloride-36 (all 1.2 μc). At the end of the incubation the discs were blotted and treated as follows. A 9.0 cm sheet of Whatman No. 1 filter paper was placed on a sheet of plate glass and wetted evenly with 1 ml of deionized water. The 12 discs from one of the reaction mixtures were blotted on each side briefly and gently, after which 4 groups of 3 discs each were extracted with 0.2 ml 95 % ethanol (ca. 3 vol). Aliquots of the ethanol extracts were applied to planchets and counted for determinations of the total cpm taken up by the discs. The cpm in the ethanol extracts are a measurement of uptake into the free space. As demonstrated later in the paper, cpm absorbed by active uptake, with the relatively high solute concentrations used, were insignificant during the 20-minute experiments. From these data and assay of the cpm per unit volume in the ambient solution at the end of 20 minutes the percent free space could be calculated. Since diffusion equilibrium was reached the same cpm/μl would occur in the ambient solution and free space.

Another method for calculation of the percent free space utilized the following data from the duplicate reaction mixtures: the volume of the incubation solution, the initial cpm/μl ,the volume (1 mg fr wt = 1 μl) of the 12 discs and the cpm/μl of reaction mixture at the end of the 20-minute incubation. This measurement was based on the principle of dilution of radioactivity in the ambient solution due to diffusion of radioactive solute into the free space of the tissue discs. Further details of these 2 methods are given later in this paper.

Solute Uptake. Uptake as used herein refers to labeled solute removed from the medium and retained by the tissue after a 1-hour washing, measured in an ethanol extract of tissue discs.

Fifteen discs (prepared as described above) were incubated in 0.5 ml (pH 7.0) of mixed L-amino-U-^{14}C acids (algal protein hydrolysate) or in various concentrations (0.00012–0.05 M) of DL-leucine-1-^{14}C or L-phenylalanine-U-^{14}C (all 1.2 μc) for 3 hours at 29° with gentle shaking. The discs were washed for 1 hour in running tap water, blotted gently and placed into test tubes in 4 volumes of 95 % ethanol, stoppered tightly and extracted in a shaker for 6 hours or more. Aliquots of the ethanol extracts were counted on planchets for determination of uptake.

Incorporation of Amino Acids. The same batches of discs used for assay of uptake at 3 concentrations of labeled leucine or phenylalanine were used for studies of amino acid incorporation (see above). After removal of aliquots of the ethanol extracts of the discs for assay of uptake, the discs were ground in 80 % (v/v) ethanol in a Potter-Elvejhem tissue homogenizer. The homogenate was centrifuged at 10,000 × *g* for 15 minutes and the precipitate washed 3 times with 10 ml of ice cold 5 % trichloroacetic acid. For the first wash the solution contained un-

labeled leucine or phenylalanine to 0.03 M. The final precipitate was incubated in 0.75 ml 0.3 N KOH for 18 hours at 37°, centrifuged at 10,000 × *g* for 15 minutes and the precipitate washed twice with 0.3 ml of water. The 3 supernatant fractions were combined and aliquots were counted on planchets in a gas flow counter for assay of radioactivity incorported.

In separate experiments, the procedure following was used to ascertain the percentage of the radioactivity in the 80 % ethanol insoluble fraction that was in protein. After incubating tissue discs in presence of labeled amino acid the discs were homogenized in 80 % ethanol, and the precipitate extracted successively with 80 % ethanol, anhydrous methanol, methanol-formic acid (0.05 M), cold 5 % trichloroacetic acid, 95 % ethanol, absolute ethanol, ethanol-ether (2:1, v/v) and anhydrous ether. The final precipitate was incubated in 0.3 N KOH at 37° for 16 hours and centrifuged as described above. By assaying radioactivity in the precipitate, and in the supernatant fraction both before and after acidification to pH 2.0 with perchloric acid it was determined that 83 % of the radioactivity incorporated was in protein.

Leakage of Amino Acids. Randomized 1.5 g batches of cannula sections (5 × 10 mm) in duplicate were prepared daily and homogenized in 4 volumes of 95 % ethanol in a Potter-Elvejhem homogenizer and centrifuged at 10,000 × *g* for 15 minutes. The supernatant fraction was assayed for Kjeldahl nitrogen as described above. Other duplicate batches of cannula sections were washed in 30 ml of deionized water for 2 hours in 125 ml flasks with gentle shaking and similarly treated and assayed. The mg of Kjeldahl nitrogen were multiplied by the factor 6.25 to convert to mg amino acid. Differences in the levels of amino acids between unwashed and washed tissue provided an indication of the rate of leakage of amino acids as a function of ripening.

Results

If banana tissue undergoes changes in membrane permeability during ripening (2, 23), it might be expected that the volume of the free space would increase to include the protoplasmic (both cytoplasm and vacuole) volume. To examine this postulate quantitatively 12 tissue discs (256 mg fr wt) were incubated in relatively high concentrations of radioactive mannitol (0.1 M), sucrose (0.03 M), fructose (0.06 M) and potassium chloride (0.07 M) for 20 minutes. This provided ample time for attainment of diffusion equilibrium of solutes throughout the ambient solution and free space.

In column 1, table I the percent free space is shown for banana discs from 10 different bananas over a 10-day period of ripening. The Q CO_2 is given for each banana at the time tissue discs were removed for assay (see also fig 1). The calculation of percent free space for column 1 is based on the principle of dilution of cpm in the ambient solution due to diffusive movement of solutes into the free space. Calculations were made as follows: initial cpm in ambient solution (A) divided by cpm/μl of ambient solution at the end of 20 minutes (B) equals the μl volume in which radioactivity is distributed (C). From the latter (C) is subtracted the initial volume (400 μl) of the solution (D), giving the μl of free space. The latter divided by volume (256 mg fr wt = 256 μl) of the 12 discs (E) times 100 gives the percent free space. The data (table I, column 1) illustrate a significant increase in free space about 1.5 days before the onset of the respiratory rise.

Table I. *Changes in Free Space of Banana Tissue to 0.1 M ^{14}C-Labeled Mannitol During Ripening*

Days from start	Fruit*	μl CO_2/ g fr wt/hr	1 % Free space based on dilut. of cpm in ambient solution	2 % Free space based on cpm in ethanol extracts of discs
			Mean SE	Mean SE
1	A	25	...	20.6 ± 0.0
2	B	25	22.0 ± 2.0	20.2 ± 0.5
3	one-half of C	25	20.0 ± 0.0	21.0 ± 0.4
4	one-half of C	25	22.1 ± 0.2	20.8 ± 0.4
5	one-half of D	25	21.1 ± 0.4	22.7 ± 0.1
6	one-half of D	25	25.4 ± 1.2	27.0 ± 0.5
7†	E	25	29.7 ± 0.4	25.2 ± 0.4
8	F	30	35.6 ± 0.4	29.8 ± 0.1
9	G	43	50.8 ± 3.1	32.8 ± 0.3
10	H	93	65.0 ± 3.6	88.5 ± 0.8
	I	93	65.1 ± 1.3	81.8 ± 0.9
	J	104**	97.0 ± 1.0	...***

* For each of the individual fruits designated by letters the Q CO_2 was assayed before tissue discs were removed for the experiments. In some instances one-half of a fruit was used each of 2 successive days.

** Climacteric peak was at 107 μl/g per hour. Banana J assayed 6 hours past peak (see fig 1).

*** No measurements obtained due to damage to discs.

† Onset climacteric.

In the second part (table 1, column 2) of the experiment the 12 discs in one of the 2 reaction mixtures were carefully blotted and 4 groups of 3 discs each were extracted with 0.2 ml (ca. 3 vol) of 95 % ethanol in a shaker, and aliquots assayed for radioactivity. Free space was calculated as follows: total cpm in discs divided by cpm/μl of ambient solution at 20 minutes equals μl free space. The latter divided by the volume (64 mg fr wt = 64 μl) of the 3 discs times 100 gives the percent free space.

Figure 1 illustrates plots of the Q CO_2 and percent free space during banana ripening. The latter is a plot of the 95 % confidence intervals for the daily means of quadruplicate samples of discs (data from column 2, table I). From these results also it is clear that an increase in free space precedes the onset of the respiratory rise by about 2 days. The percent free space calculated from these data obtained from ethanol extracts is in close agreement day by day wtih the percent free space determined on basis of dilution of cpm in the ambient solution, except for a slight disparity during the climacteric rise. Part of the basis for the lower values shown for this period in column 1 (table I) is greater self-absorption due to leakage of solutes into the ambient solution. Also the physical state of the tissue during the climacteric, due to loss of turgor and partial separation of cells, made it difficult to blot discs uniformly.

At the molarity of mannitol used active uptake was insignificant from the standpoint of radioactivity removed from the solution. In fact there was no measurable difference, for both preclimacteric and climacteric tissues, between cpm in the ambient solution at 15 and 40 minutes after adding the discs, which indicates that diffusion equilibrium was reached and any active uptake of radioactivity was too small

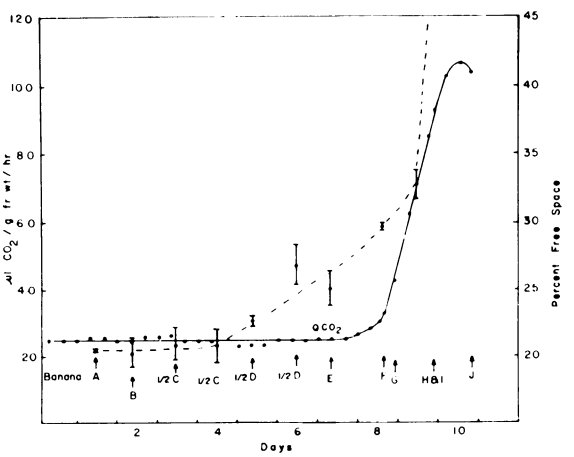

FIG. 1. Percent free space of banana tissue to 0.1 M ^{14}C-labeled mannitol during ripening (broken line) and plot of CO_2 evolution (solid line). CO_2 evolution was measured for each banana immediately before preparation of tissue discs. The data for percent free space are from table I (part II) and show the 95 % confidence intervals for daily means of quadruplicate samples of 3 discs.

Table II. *Solute Uptake During Banana Ripening*

Reaction mixtures (0.5 ml) containing solute as indicated (1.2 μc), 0.03 M phosphate buffer, pH 7.0 and 320 mg fresh weight of tissue discs were incubated for 3 hours at 29° in a shaker. The tissue was washed 1 hour in running tap water, extracted with 4 volumes 95 % ethanol and aliquots of the ethanol extracts counted on planchets.

	cpm Uptake		
Days from start	Expt I Mixed amino acids 1.7 μg/ reaction mixture	Expt II Phenylalanine 0.001 M	Expt III Leucine 0.001 M
1	46,500	...	...
2	57,500	...	...
3	57,000	...	...
4	46,200	...	45,000
5	49,200	...	...*
6	51,000	31,200	21,500
7**	38,200	17,200	14,530
8	27,900	2040	12,050
9	3400	1710	11,070
10	...	...	5800

* No measurements made.
** Onset climacteric.

to have a significant effect on the accuracy of the measurements of free space. Similar trends in increase in free space were observed with 0.03 M sucrose, 0.06 M fructose and 0.07 M potassium chloride as the radioactive solutes. The increases in free space, measured at diffusion equilibrium, indicate a progressive increase in the proportion of cells which has become totally permeable to solutes by simple diffusion, rather than the extent of permeability of all the cells of the tissue.

Active Uptake. Discs were incubated for 3 hours in radioactive solutions of amino acids, washed for 1 hour and extracted with ethanol. Uptake was assayed in the ethanol extracts as the cpm retained after washing, which removes solutes from the free space (whether cell wall region, or the intracellular volume of cells which had become totally permeable to solutes by diffusion). It was necessary to use this criterion of active uptake, namely, the capacity to take up and retain solutes, in order to distinguish from passive uptake due to diffusive movement into cells which have become free space, which would be included were uptake measured as the removal of label from the external solution.

There occurred a marked reduction in active uptake (table II) prior to the onset of the climacteric, and during the period of the respiratory rise active uptake had fallen to a low level (from 5–13 % of preclimacteric level). The implication of the results is that active uptake diminishes owing to a progressive increase in the fraction of cells totally permeable to solutes. Solutes entering these cells are readily

lost during the washing period. Similar results were obtained when the exogenous concentration of leucine or phenylalanine ranged from 0.005 to 0.05 M, and when potassium chloride was the exogenous solute. The diminution in capacity for active uptake during ripening parallels the progressive increase in free space reported above.

Leakage of Amino Acids. A correlation between the increased rate of leakage during the climacteric and an increase in sugar content of bananas during ripening has been interpreted to mean that the permeability of the tonoplast to sugar remains constant (9). In the present study it could be demonstrated that there is no significant change in 80 % ethanol soluble Kjeldahl nitrogen (which includes amino acids) in freshly cut, unwashed tissue during ripening (fig 2). In contrast, in tissue sections which had been washed for 2 hours in water prior to assay there occurred a large decrease (61 % at the climacteric peak) in ethanol soluble Kjeldahl nitrogen (fig 2). This fraction would contain small polypeptides as well as free amino acids. Thus, it is probable that a portion of the nitrogen remaining after the washing occurs in the form of small polymers, which do not readily leak through cellular membranes during the washing, even though alterations in membrane properties had occurred. If this were so, then the rate of leakage of amino acids would be greater than the data in figure 2 indicate. The difference between the levels of ethanol soluble Kjeldahl nitrogen in washed and unwashed tissue at the start of the experiment is attributable largely to materials washed from cut and injured cells at the periphery of the sections, and to a lesser extent to a basal level of leakage.

From these and the previous results (23), it is clear that increased leakage of solutes (sugar or amino acids) is directly related to changes in permeability and not simply to an increase in levels of endogenous solutes. By use of other methods it has been shown that there is an increase in salt leakage during the climacteric in banana tissue (2).

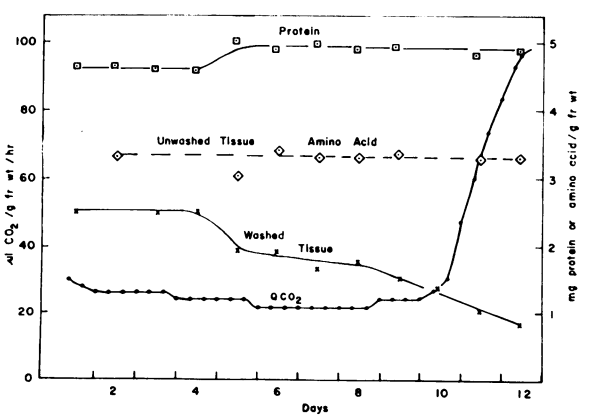

FIG. 2. Plots of CO_2 evolution (•), protein levels (□), and amino acid content of unwashed tissue (△) and tissue washed for 2 hours (×) during ripening of bananas.

Amino Acid Incorporation and Pool Size at the Site of Protein Synthesis. The several manifestations of changes in permeability during ripening of bananas indicated that normal aspects of protoplasmic compartmentalization would be impaired during, and just prior to the climacteric. From the onset of changes in permeability to the climacteric peak the cpm incorporated represent a complex of incorporation within (A) that proportion of the tissue volume which has essentially normal permeability properties and (B) the remaining cell population which is completely free space. During the climacteric practically the entire tissue volume becomes free space to solutes in the ambient solution, and endogenous amino acids leak readily into the ambient solution. Thus, it could be expected that there would occur an approximate equilibration of unlabeled, endogenous amino acid and labeled, exogenous amino acid throughout the volume of solution and tissue. As a result the specific activity of the substrate at the site of protein synthesis would be altered, dependent upon the relative amounts of the 2 pools of substrate. The higher the concentration of the labeled substrate in the ambient solution the smaller would be the effect of unlabeled endogenous amino acid in diluting the specific activity of the pool at the site of protein synthesis.

An analysis of relative changes in compartmentalization and pool size at the site of protein synthesis and an approximation of the rate of amino acid incorporation at the climacteric peak as compared with the preclimacteric phase may be derived from the data presented in table III. For these experiments tissue discs were incubated in 3 concentrations of ^{14}C-labeled DL-leucine or L-phenylalanine (0.00012–0.05 M) for 3 hours, washed for 1 hour, extracted with 4 volumes of 95 % ethanol and assayed for radioactivity in protein and in the ethanol soluble extract. It was determined for tissue with normal permeability properties that an exogenous concentration of 0.05 M was saturating for uptake and incorporation.

For each of the 3 concentrations of exogenous leucine (expt I, table III) there occurred a decline in μg incorporated proceeding from the preclimacteric phase to the climacteric peak, with the ratios of μg incorporated at the preclimacteric phase/climacteric peak diminishing with increase in substrate concentration. Therefore, the ratios indicate largely the relative effect of dilution of the radioactive leucine pool at the site of amino acid incorporation by a previously sequestered endogenous pool, rather than decreases in the rate of incorporation. That the ratios diminish with increasing concentration of exogenous substrate is due to the lesser effect of the endogenous pool in diluting, and thus lowering the specific activity of the pool at the site of protein synthesis. This interpretation provides an explanation also for the fact that the ratios of μg incorporated at 0.001/0.005 M diminish proceeding from the

Table III. *Amino Acid Incorporation During Banana Ripening*

Expt	320 mg discs in 0.5 ml	μg Incorporated*				
		Pre-climacteric	Onset	Rise (middle)	Peak of climacteric	Ratio of pre/peak
I	Leucine					
	0.00012 M (8 μg)	0.52	0.34	0.011	0.015	35.0
	0.001 M (65 μg)	0.97	0.14	0.095	0.092	10.6
	0.005 M (325 μg)	1.44	0.45	0.35	0.40	3.6
	Ratio 0.001/0.005 M	(0.68)	(0.31)	(0.27)	(0.23)	
II	Phenylalanine					
	0.001 M (82 μg)	0.96	0.51	0.27	0.19	5.0
	0.005 M (412 μg)	1.64	1.30	0.88	0.72	2.3
	0.05 M (4120 μg)	11.20	8.70	6.60	5.90	1.9
	Ratio 0.001/0.005 M	(0.59)	(0.39)	(0.31)	(0.26)	
	Ratio 0.005/0.05 M	(0.15)	(0.15)	(0.13)	(0.12)	

* For calculation of μg incorporated from cpm incorporated the specific activity of the exogenous solute (cpm/μg) was used. Data in parentheses are calculated ratios (not μg incorporated). The figures in parenthesis next to the substrate molarities are μg substrate/ reaction mixture.

preclimacteric period to the respiratory peak. Were there no differential changes in specific activity of the metabolic pools during ripening (due to changes in compartmentalization) these ratios would remain close to constant.

The data for phenylalanine incorporation (expt II, table III) show the same trend as for leucine for both types of ratios, even though obtained from experiments with tissues from a different hand of bananas. The lower magnitude (ca. one-half) of the Pre/Peak ratios (at exogenous concentrations of 0.001 and 0.005 M) for phenylalanine than for leucine could indicate a smaller endogenous pool of phenylalanine and thus a lesser effect thereof in dilution of the labeled pool at the site of amino acid incorporation. In this experiment a much higher concentration of labeled substrate (0.05 M or 4120 μg/reaction mixture) was used to provide an amount too large to be significantly affected by dilution by the endogenous, unlabeled pool at the site of protein synthesis. The fact that the ratio of μg incorporated at 0.005/0.05 M phenylalanine is essentially constant during the process of ripening indicates no significant effect of unlabeled phenylalanine in diluting the metabolic pool. Thus, the specific activity of the metabolic pool remained close to constant from the preclimacteric period through the climacteric peak. In view of this, the ratio (1.9) of μg incorporated at the preclimacteric/climacteric peak (for 0.05 M conc) may indicate an approximate halving of the rate of phenylalanine incorporation at the climacteric peak. Since during this period there is no significant decline in protein level (fig 2), it would appear that the rate of protein degradation is rather low.

In another experiment replicates of discs (320 mg fr wt) from climacteric tissue were incubated for 3 hours in 0.5 ml volumes of 0.0001 M, 0.001 M and 0.005 M L-phenylalanine, whereas 3 other replicates of discs were prewashed for 1 hour in 10 ml of water, blotted and similarly incubated. The cpm incorporated for the unwashed discs in the 3 concentrations of phenylalanine respectively were 835, 692 and 845 (0.013, 0.11 and 0.67 μg incorporated respectively), while for the washed discs the cpm incorporated were 2340, 1530 and 1520 (0.038, 0.24 and 1.2 μg incorporated respectively). The results are about what could be expected for a tissue in which endogenous amino acids are readily leached. The increased incorporation in washed discs may be attributed to leaching out of unlabeled phenylalanine and thereby diminishing dilution of the labeled pool during the subsequent incubation.

Discussion

The results of the present study indicate that permeability changes precede the onset of the climacteric respiratory rise in bananas. By the use of 2 methods of assay it could be demonstrated that free space increases significantly about 36 to 44 hours before the onset of the climacteric (table I, fig 1), and that by the respiratory peak the tissue is close to 100% free space. For firm, green preclimacteric tissue the free space (ca. 20%, table I) is probably confined largely to the cell wall region. Since measurements of free space were made at diffusion equilibrium, the increase in free space is attributed to a progressive increase in the proportion of cells which has become totally permeable to solutes in the ambient solution by simple diffusion. Associated with the increase in free space there occurs a marked decline in the capacity of the tissue to take up and retain solutes (table II), and an acceleration in the rate of leakage of amino acids (fig 2). Thus, the conclusion (9) that the permeability of banana tissue remains constant during ripening is not tenable.

From data on amino acid incorporation in presence of 3 concentrations of exogenous substrate (table III) it could be deduced that changes in membrane

permeability during ripening result in alterations in cellular compartmentalization and size of the amino acid pool at the site of protein synthesis. For low concentrations of exogenous substrate the decline in μg of amino acid incorporated during ripening is more apparent than real, and may be regarded largely as the effect of a gradual increase in the number of cells which have become completely permeable to the substrate. Under such conditions there occurs a diffusive mixing of unlabeled, vacuolar amino acids with exogenous, labeled amino acid at the site of protein synthesis, resulting in a marked lowering of the specific activity of the metabolic pool. The large decline in the ratios of μg amino acid incorporated during the preclimacteric to the climacteric period, with increase in concentration of exogenous substrate, is explicable in terms of a decrease in the diluting effect of the unlabeled pool on the specific activity of the metabolic pool.

Theoretically, with a high concentration of exogenous, labeled substrate at the climacteric peak, the specific activity of the substrate at the site of protein synthesis would not change appreciably attending a diffusive mixing of labeled and unlabeled pools, due to the small diluting effect of the endogenous, unlabeled pool. These conditions were achieved experimentally by incubating 320 mg of discs in a 0.5 ml solution containing 4120 μg of L-phenylalanine-U-^{14}C. Kjeldahl nitrogen (mg) in the 80 % ethanol soluble fraction of the tissue converted to 1.05 mg of amino acid/320 mg of tissue discs. If all of this were free amino acids (although unlikely, as some would occur in the form of small polypeptides), the amount of phenylalanine could not be expected to be more than 10 % of the total, or about 105 μg. Since the endogenous, unlabeled pool of amino acid is less than 2.5 % of the size of the exogenous, labeled pool, the mixing of these pools at the site of protein synthesis would have no significant effect on the specific activity. Thus, the approximately 50 % reduction in μg of phenylalanine incorporated (table III) during the climacteric peak as compared with the preclimacteric period may indicate a similar decline in the rate of protein synthesis. Considering that there is no decrease in the level of protein (fig 2), it would appear that the rate of protein degradation is relatively low.

Since an increase in ethylene content precedes the onset of the respiratory rise in banana by 3 hours (8), and significant changes in permeability occur about 40 hours earlier, it would appear that enhanced ethylene production results from changes in permeability. Some investigators have previously regarded the increase in ethylene production as a product rather than a cause of climacteric changes (5). Addition of ethylene accelerates the onset of the climactic, but has no significant effect on the respiratory rate (6). Ethylene has been shown to increase membrane permeability of fruit mitochondria (16). Thus, the possibility is presented that alterations in protoplasmic compartmentalization cause an increase in ethylene synthesis, which later in turn contributes to an acceleration of the deterioration of the properties of cellular membranes. This is consistent with the observed rapid acceleration of changes in free space during the respiratory rise (fig 1).

Although permeability changes occur during senescence of nonclimacteric (e.g., bean endocarp, 22) as well as climacteric fruit tissues, the cause-effect relationships may differ. For example, in bean endocarp there occurs a large decline in RNA, followed by degradation of DNA and protein, all declining 25 to 40 % before the onset of measurable changes in properties of cellular membranes (24, 25). In contrast, in a climacteric fruit (banana) there is no evidence of degradation of such macromolecules before the onset of permeability changes, at least based on assays of total protein (fig 2). Not enough experimental data is available to provide an explanation for the fact that auxin prevents permeability changes in a nonclimacteric fruit through a more primary effect on RNA and protein synthesis (25), while addition of auxin accelerates the onset of senescence in banana (10).

A number of chemical changes have been associated with the climacteric of ripening fruits (1, 6, 17, 18, 19, 20, 26), some of which have been suggested as causative. Possible cause-effect relations between permeability changes and other climacteric phenomena have been discussed previously (23). Since the climacteric is preceded by permeability changes the latter may be construed as causative, and also as a factor which should be considered in interpretations of other phenomena associated wtih the climacteric.

Acknowledgment

I thank Mr. A. J. Kowalski for valuable technical assistance, and McCann Produce Company, Los Angeles, for providing green bananas.

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