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Polyribosomes from Pear Fruit

CHANGES DURING RIPENING AND SENESCENCE

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ABSTRACT

Polysome profiles were examined from lyophilized peel tissue of ripening pear (*Pyrus communis*, L. var. Passe-Crassane). Messenger RNA chains bearing up to eight ribosomes (octamers) were resolved and exhibited the highest absorption peak when ribonuclease activity was eliminated during extraction. Neither normal ripening nor the increase of large polyribosomes that normally accompanies ripening and senescence of the fruit occurred when pretreatment at 0 C was omitted. Normal ripening and increase of large polyribosomes would, however, be initiated by an ethylene treatment. The size distribution of the polyribosomes remained essentially constant throughout a 4-month cold storage; there was, however, a large increase in ribosomes by the 12th week of storage.

Detailed analyses of polysome distributions were used to measure the amount of polysomal associated mRNA present in tissues of different stages of ripening and senescence. These analyses led to the further conclusion that two different phases of senescence (ripening) can clearly be distinguished; for each of these two phases, a similar sequence of events was found, *i.e.* an increase in mRNA followed by a constant level of mRNA, possibly paralleled by an increase in initiation. During normal ripening, the initial increase in mRNA occurred during the preliminary period of cold storage. The lack of this preripening storage at 0 C was closely related to a decrease in large mRNA and a loss of larger polyribosomes while the usual sequence of events was restored by an ethylene treatment.

At the beginning of the climacteric phase, a marked increase in the protein nitrogen content of apples has been reported (11, 12) and it is now well established that an enhanced protein synthesis may be linked to maturation (16–18). In the case of the variety of pear fruit studied in our laboratory (*Pyrus communis* L. var. Passe-Crassane), an optimal period of 12 weeks of cold storage prior to submitting the fruit to 15 C for ripening is needed for a normal ripening, *i.e.* fruits exhibiting the characteristic climacteric respiratory pattern. This climacteric rise does not occur when pear fruits are placed immediately at 15 C after the harvest; however, it can be induced by an ethylene treatment (14).

In an earlier paper (4) we showed that high yields of large polyribosomes were obtained from lyophilized peel tissue of pear, by using a Tris-HCl buffer at high concentration (0.4 M) and pH (8) as reported in studies with different tissues (1, 3, 15, 17). In this report, this method was used to examine the polyribosome distribution of peel tissues of fruits ripened in different conditions, in order to determine whether this distribution changed during ripening and senescence and whether these changes were typical of either climacteric or nonclimacteric respiratory pattern, or both. In addition, changes in the rate of initiation (attachment) of ribosomes onto mRNA and variations in the amount of available mRNA were estimated for different stages of ripening and senescence.

MATERIALS AND METHODS

Fruits. Pears (*P. communis*, L. var. Passe-Crassane) harvested at the beginning of November were stored either at 0 C (normal ripening) or at 15 C (abnormal ripening). Fruits stored at 0 C during 12 weeks were subsequently transferred to 15 C for ripening while fruits stored initially at 15 C were placed either in air or in air with ethylene (500 μl/l; continuous flow).

Measurement of Respiratory Rates and Preparation of Samples. Representative samples of 15 fruits selected for uniformity of size and color were placed in respiration jars and supplied with continuous air flow. The CO_2 was measured daily with an IR analyzer Onera model 80. At each indicated time (see Figs. 2–5), five fruits from a jar were quickly peeled and peel tissues were immediately frozen in liquid N_2, then lyophilized and mixed in an appropriate storage bottle for subsequent isolation of ribosomes. The skins were taken from the entire fruit always with the same potato peeler in order to obtain peel of reasonably uniform thickness. Microscopic controls have previously shown the same proportion of mesocarp in peel tissues (6).

Polysome Extraction Procedure. The procedure, as previously described (4), was conducted at 0 to 4 C. Extraction was performed on 5 g of pulverized material by homogenizing in 25 ml of cold extraction solution (buffer A: 0.4 M Tris [pH 8], 11 mM mercaptoethanol, 3% PEG, 60 mM KCl, 35 mM $MgCl_2$, 6% sucrose, and 2.5 g Polyclar). The resulting brei was clarified by centrifugation for 10 min at 15,000*g* and the supernatant was gently layered over 4 ml of 1.2 M sucrose (in buffer B: 10 mM KH_2PO_4 [pH 8], 10 mM $MgCl_2$) and spun for 90 min at 105,000*g* (average in the R_{50} rotor of a Spinco model L ultracentrifuge). The pellet was rinsed gently and resuspended in buffer B up to 0.5 ml by means of a close fitting Teflon pestle. Aliquots (0.2 ml) of resuspended polyribosomes issued from equivalent amounts of tissue were layered onto linear (15–60%, w/v) sucrose gradients in buffer C: (40 mM Tris-HCl [pH 8.5], 10 mM $MgCl_2$, 20 mM KCl) and spun for 45 min at 50,000 rpm in a SW 60 rotor. The gradients were prepared by layering 1.9 ml of sucrose at 62% in cellulose nitrate tubes followed by 1.9 ml of sucrose at 13% and equilibrated for 4 h at 2 C (20).

Polysome Profiles Analysis. After density gradient centrifugation, the contents of the tubes were analyzed with an ISCO model 185 density gradient fractionator attached to an ISCO model UA5 absorbance monitor. The areas in different regions of the polysomal profiles were determined from the average of three measurements of two different curves by counting the number of squares of the area under each profile peak with an accuracy of 2.5%. The different regions were: subunits, monosomes, polyribosomes, and large polyribosomes sedimenting faster than pen-

tamers. Equilibrated blank gradients were always monitored in order to report the base line for each figure, and the area below is excluded from calculations. The areas under different peaks were divided by the number of ribosomes in polysomes of that size class. Thus, the values represent the relative contents of mRNA strands in each polymeric class. This analytical approach of Davies and Larkins (2) rests upon several assumptions. First, the *A* of mRNA should be negligible compared with the *A* of the ribosomes. Second, none of the monosomes must be attached to short mRNA species. Third, the area of *A* of each class size of unresolved (larger) polyribosomes must be approximated; a 10-mers was used as the average size class. Fourth, RNase activity must be minimal and only intact polysomes (mRNA) can be present. As it was observed that large polysome size classes were completely absent from different profiles (Fig. 2, A, B, and C, for example), assays were conducted to determine whether polysome degradation occurred during the extraction procedure. RNase activity is determined by the conversion of large polysomes to small polysomes. Tissues exhibiting no large polysome were mixed with postclimacteric tissues (with large polysomes present, Fig. 2E) and extracted together; large polysomes size classes were not degraded. These results demonstrate that the inability to isolate large size classes from these tissues was not due to the activity of RNase during extraction.

RESULTS

The following values, related to the 1977 harvest, were determined from the average of three different polysome extractions. Similar results have been found for the two previous harvests.

Cold Storage followed by Normal Ripening. After 12 weeks of cold storage, pear fruit undergoing normal ripening at 15 C exhibited the usual climacteric respiratory pattern with a climacteric peak after 11 days always followed by another respiratory rise on day 18 (Fig. 1). This respiratory curve, a characteristic of ripening Passe-Crassane pears, has been described in previous work (6). Representative profiles of the polyribosomes at different stages of cold storage followed by normal ripening were obtained (Figs. 2 and 3, respectively). The surface areas under the different

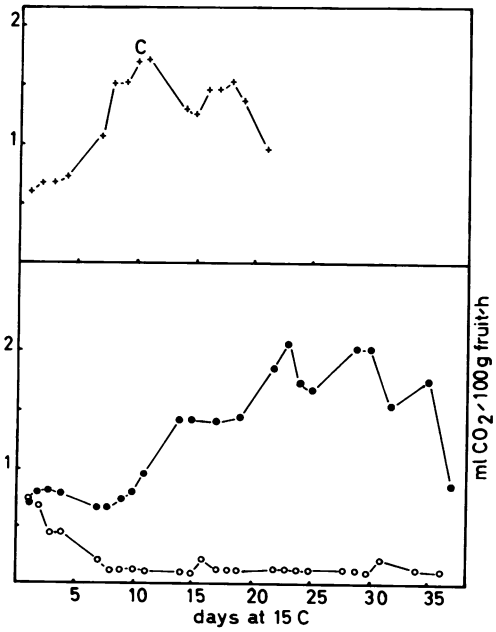

FIG. 1. Respiratory activity of pear fruit held continuously at 15 C either after 12 weeks of cold storage (+——+), or immediately after the harvest in air (O——O) or in air with ethylene, 500 µl/l, continuous flow (●——●). C: climacteric peak.

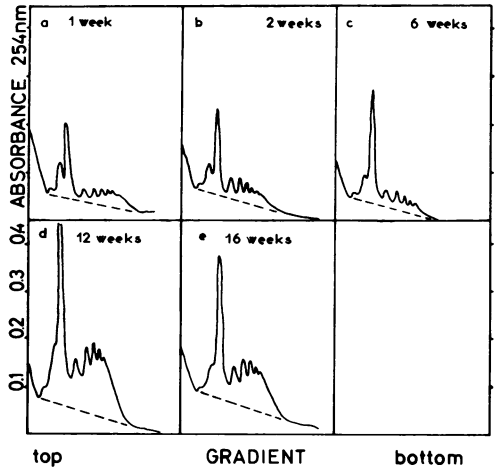

FIG. 2. Cold storage. Sucrose density gradient profiles of ribosomal population extracted from peel tissues of pear fruit held for different number of weeks at 0 C, *i.e.* a, 1 week; b, 2 weeks; c, 6 weeks; d, 12 weeks; e, 16 weeks. The periods examined represented specific stages of storage, *i.e.* a to c, prior to normal ripening; d, cold period optimal for a normal ripening; e, cold period longer than 12 weeks.

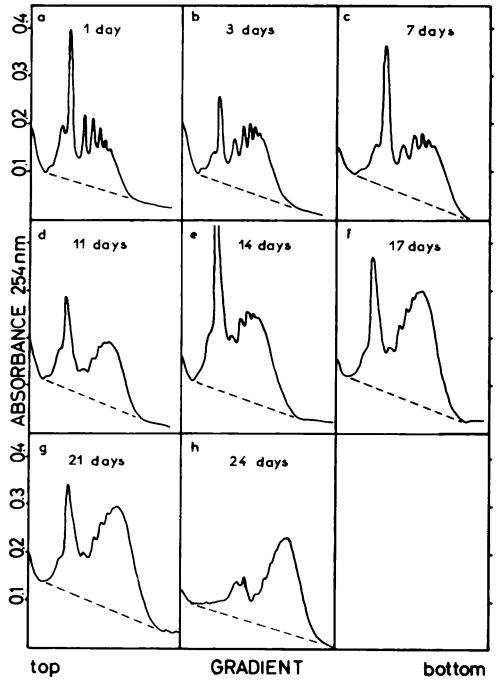

FIG. 3. Normal ripening. Sucrose density gradient profiles of ribosomal population extracted from peel tissues of pear fruit held for different numbers of days at 15 C after 12 weeks of cold storage, *i.e.* a, 1 day; b, 3 days; c, 7 days; d, 11 days; e, 14 days; f, 17 days; g, 21 days; h, 24 days. The periods examined represented specific stages of ripening and senescence, *i.e.* a to c, preclimacteric period; d, climacteric peak; e to h, postclimacteric period.

portions of the profiles depicted in Figures 2 and 3 are given in Tables I and II.

The proportion of large polyribosomes which remained essentially constant (about 36%) during cold storage increased throughout ripening and senescence while the proportion of monosomes decreased from 55 to 19%. The increase in large polysomes and decrease in monosomes suggest that one of two processes may be occurring during ripening and senescence. There is either an increase in the number of available mRNA templates (especially the one coding for larger proteins), or there is an increase in the

rate of initiation of ribosomes onto mRNA (22) or both. The total amount of mRNA and ribosomes increased quickly after 12 weeks of cold storage and after 14 days of ripening at 15 C, just after the climacteric peak (Table II). Before the first increase in mRNA no change in the polysomal population was detected while each increase in mRNA and ribosomes (monosomes) was followed by a progressive increase in larger polysomes. The increase of 47 units of monosomes after the climacteric peak (day 14) was followed on day 17 by an increase of 44 units of large polysomes (≥ 7 mers). These results strongly suggest an increased rate of initiation of ribosomes onto mRNA.

Consequently, the following sequence of events has been found during cold storage and normal ripening: (*a*) an increase in mRNA and ribosome production after 12 weeks of cold storage, followed by possible enhancement of initiations; (*b*) a second increase in mRNA and ribosome production after the climacteric peak followed by another enhancement of initiations prior to a decrease in mRNA and ribosomes. After 16 weeks of cold storage, there was a lower amount of ribosomal material and mRNA which suggests that ribosomes and mRNA synthesis did not keep pace with a longer cold storage (Tables I and II).

The increase in polyribosomes noted with progressive ripening is contrary to two other studies with senescing (ripening) Bartlett pear fruit (13–17). Differences in results may be related to difference in fruit variety and tissue; Romani and his collaborators studied pulp tissues.

Table I. *Changes in Polyribosomal Distribution Accompanying Cold Storage followed by Normal Ripening and Abnormal Ripening With or Without Any Ethylene Treatment*

Data were taken from profiles depicted in Figures 1 through 4.

Time and Ripening	Total Ribosomal Material	L/P[a]	M/T[b]	P/T[c]
	arbitrary units		%	
Cold storage				
1 week at 0 C	53	41	55	45
2	49	36	56	44
6	56	36	66	34
12	178	35	46	54
16	118	37	46	54
Normal ripening				
1 day at 15 C	141	27	45	55
3	127	41	26	74
7	173	35	40	60
11	154	50	34	66
14	257	45	39	61
17	282	59	25	75
21	270	59	25	75
24	187	63	19	81
Abnormal ripening without ethylene				
1 day at 15 C	48	37	42	58
9	150	42	53	47
16	144	56	36	64
25	108	26	65	35
32	95	23	73	27
37	112	35	60	40
43	150	40	50	50
Abnormal ripening with ethylene				
4 days at 15 C	113	35	43	57
11	247	39	49	51
14	253	46	35	65
25	258	53	31	69
32	340	76	12	88
37	200	73	16	84

[a] Large polysomes (>5 mers) as a percentage of total polysomes.

[b] Monosomes as a percentage of total material (monosomes + polysomes).

[c] Polysomes as a percentage of total material (monosomes + polysomes).

Abnormal Ripening. Without prior storage at 0 C some ripening, albeit abnormal, takes place. For example, some changes typical of ripening, like color change, occur while others also typical of ripening, like softening and volatiles emissions, do not.

Pear fruit stored in air at 15 C immediately after the harvest did not exhibit the usual climacteric respiratory pattern while an ethylene treatment restored a normal ripening pattern and the climacteric peak after 26 days at 15 C (Fig. 1). Representative profiles of abnormal ripening with or without any ethylene treatment were obtained (Figs. 5 and 4, respectively). The surface areas under the different portions of the profiles depicted in Figures 4 and 5 are given in Tables I and II.

An increase in the proportions of large polyribosomes at the beginning of an abnormal ripening in air was followed by a dramatic decrease from 56% on day 16 to 23% on day 32; afterwards, a new increase up to 40% occurred on day 43 (Table I). The total amount of ribosomes and mRNA varied in the same way; yet we notice that between 16 days and 32 days, the decrease of the total amount of ribosomes and mRNA is only due to a strong decrease of large polyribosomes and large mRNA species while the small polymers (≤ 3 mers) content remained relatively constant. Thus, the following sequence of events during an abnormal ripening in air at 15 C can be described: an increase in mRNA and ribosome production followed by possible increase in initiation, then a dramatic decrease in ribosome synthesis and large mRNA species followed by a new increase in mRNA and ribosome production.

An ethylene treatment restored the usual sequence of events found during cold storage followed by a normal ripening, *i.e.* a continuous increase from 35 to 73% in the proportion of large polyribosomes concomitant with a decrease from 43 to 16% in the proportion of monosomes (Table I). The sequence of events occurring under ethylene treatment, which is similar to that of

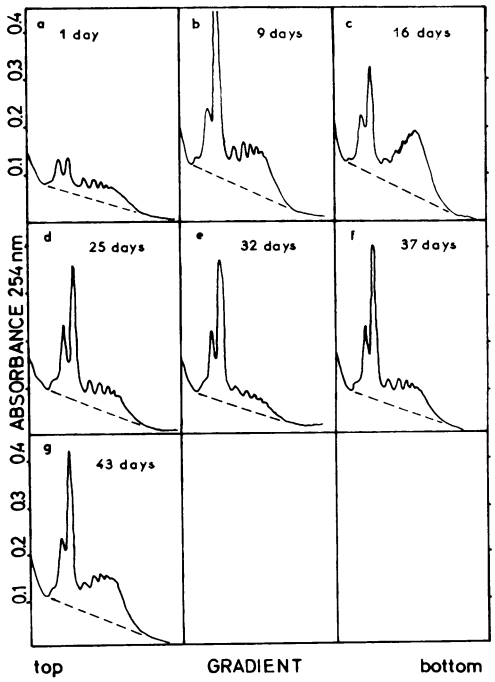

FIG. 4. Abnormal ripening without any ethylene treatment. Sucrose density gradient profiles of ribosomal population extracted from peel tissues of pear fruit held in air at 15 C for different number of days immediately after the harvest, *i.e.* a, 1 day; b, 9 days; c, 16 days; d, 25 days; e, 32 days; f, 37 days; g, 43 days.

Table II. *Analysis of Polyribosomes Distribution Accompanying Cold Storage Followed by Normal Ripening and Abnormal Ripening Without or With Any Ethylene Treatment*

Data were taken from profiles depicted in Figures 1 through 4.

No. of Ribosomes in Polysome Size Class (N)	Area (A) Peak (arbitrary units)								mRNA/peak A/N							
Cold Storage																
Weeks at 0 C	1		2	6		12	16		1		2	6	12		16	
1[a]	29		26	36		80	57		0		0	0	0		0	
2	4		5	5		17	12		2.00		2.50	2.50	8.50		6.00	
3	3		4	4		21	12		1.00		1.33	1.33	7.00		4.00	
4	4		3	3		15	9		1.00		0.75	0.75	3.75		2.25	
5	3		3	2		13	8		0.60		0.60	0.40	2.60		1.60	
6	3		3	2		13	8		0.50		0.50	0.33	2.16		1.33	
≥7	7		5	3		19	12		0.70		0.50	0.30	1.90		1.20	
Total	53		49	56		178	118		5.80		6.18	5.61	25.91		16.38	
Normal Ripening																
Days at 15 C	1	3	7	11	14	17	21	24	1	3	7	11	14	17	21	24
1[a]	61	32	63	50	97	74	66	33	0	0	0	0	0	0	0	0
2	19	14	15	13	23	19	16	12	9.50	7.00	7.50	6.50	11.50	9.50	8.00	6.00
3	16	17	23	14	22	21	25	15	5.33	5.66	7.66	4.66	7.30	7.00	8.33	5.00
4	15	15	18	13	26	29	23	19	3.75	3.75	4.50	3.25	6.50	7.25	5.75	4.75
5	9	10	13	13	19	20	20	15	1.80	2.00	2.60	2.60	3.80	4.00	4.00	3.00
6	8	12	12	13	18	23	21	18	1.33	2.00	2.00	2.16	3.00	3.83	3.50	3.00
≥7	13	27	29	38	52	96	99	75	1.30	2.70	2.90	3.80	5.20	9.60	9.90	7.50
Total	141	127	173	154	257	282	270	187	23.01	23.11	27.16	22.97	37.30	41.18	39.48	29.25
Abnormal Ripening Without Any Ethylene Treatment																
Days at 15 C	1	9	16	25	32	37	43		1	9	16	25	32	37	43	
1[a]	20	76	48	71	69	67	76		0	0	0	0	0	0	0	
2	5	12	8	8	8	8	12		2.50	6.00	4.00	4.00	4.00	4.00	6.00	
3	5	14	12	9	6	9	14		1.66	4.66	4.00	3.00	2.00	3.00	4.66	
4	4	9	12	5	4	6	9		1.00	2.25	3.00	1.25	1.00	1.50	2.25	
5	3	9	12	5	2	6	9		0.60	1.80	2.40	1.00	0.40	1.20	1.80	
6	3	9	13	4	2	5	10		0.50	1.50	2.16	0.66	0.33	0.83	1.66	
≥7	8	21	39	6	4	11	20		0.80	2.10	3.90	0.60	0.40	1.10	2.00	
Total	48	150	144	108	95	112	150		7.06	18.31	19.46	10.51	8.13	11.63	18.37	
Abnormal Ripening With an Ethylene Treatment																
Days at 15 C	4	11	14	25	32	37			4	11	14	25	32	37		
1[a]	48	118	86	78	38	31			0	0	0	0	0	0		
2	12	19	20	18	18	7			6.00	9.50	10.00	9.00	9.00	3.50		
3	11	22	27	20	20	11			3.66	7.33	9.00	6.66	6.66	3.66		
4	10	19	20	25	25	15			2.50	4.75	5.00	6.25	6.25	3.75		
5	9	19	22	22	29	16			1.80	3.80	4.50	4.40	5.80	3.20		
6	9	18	22	24	32	18			1.50	3.00	3.66	4.00	5.33	3.00		
≥7	14	32	56	71	178	104			1.40	3.20	5.60	7.10	17.80	10.40		
Total	113	247	253	258	340	202			16.86	31.58	37.76	37.41	50.84	27.51		

[a] It is assumed that no monosomes were associated with mRNA.

normal ripening, can be summarized as follows: (*a*) an increase in mRNA and ribosome production followed by possible increases in initiation; (*b*) a second increase in mRNA and ribosome production after the respiratory peak followed by a decrease in mRNA and ribosome production.

DISCUSSION

Our data show that ribosome and mRNA synthesis occurred after 12 weeks of cold storage (this preripening delay is usually needed before a normal ripening [21]). On the other hand, 16

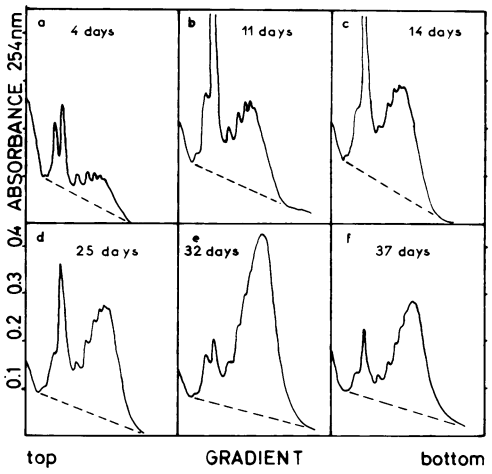

FIG. 5. Abnormal ripening with an ethylene treatment. Sucrose density gradient profiles of ribosomal population extracted from peel tissues of pear fruit held at 15 C for different numbers of days immediately after the harvest in air with ethylene, 500 µl/l, continuous flow, *i.e.* a, 4 days; b, 11 days; c, 14 days; d, 25 days; e, 32 days; f, 37 days. The periods examined represented specific stages of ripening and senescence *i.e.* a to c, preclimacteric period; d, climacteric peak; e to f, postclimacteric period.

weeks of cold storage resulted in a lower ripening ability and in a lower amount of ribosomal material and mRNA. So, an optimum ripening ability seems to be closely linked to a larger amount of ribosomes and mRNA.

Two different phases of senescence (ripening) can be distinguished: before the climacteric peak, an increase in mRNA is followed by a constant level of mRNA and possible increases in initiation; then, just after the climacteric peak, a similar sequence is initiated. Increases in mRNA content resulted in more mRNA present in each class of ribosomes (monosomes to ≥ 7 mers) whereas increases in initiation rate resulted in larger amounts of large mRNA species (especially the ones coding for larger proteins [19]). During normal ripening and senescence larger protein synthetic capacity exhibited two progressive increases reaching two maxima: one, just before the climacteric peak; and the second, during the postclimacteric period. A large protein (NADP-malic enzyme) which has a mol wt of 224,000 daltons (10) probably plays an important role in the ripening of pear fruit. Its activity rises to a maximum at the climacteric maximum (9) and this increase is accompanied by a synthesis, *de novo*, of the enzyme (7, 8). A lack of cold storage leads to an abnormal ripening and to the loss of large mRNA species at the same time that the maximum of malic enzyme activity is also lost (8). An ethylene treatment restores the normal ripening process (14) and the two maxima of the larger protein synthetic capacity; it also restores the maximum of malic enzyme activity (8). Thus, we can speculate that the ripening process may be related to the preclimacteric increase in larger protein synthetic capacity and to NADP-malic enzyme synthesis; on the other hand, the second increase of large mRNA species can be related to: (*a*) the overripening process when fermentation and brown tissues occur; (*b*) the pyruvic decarboxylase synthesis (an increased activity of this large protein has been found just after the climacteric peak [5]).

LITERATURE CITED

1. BREEN MD, EI WHITEHEAD, DG KENEFICK 1971 Requirements for extraction of polyribosomes from barley tissue. Plant Physiol 49: 733–739
2. DAVIES E, BA LARKINS 1972 Polyribosomes from peas. II. Polyribosome metabolism during normal and hormone-induced growth. Plant Physiol 52: 339–345
3. DAVIES E, BA LARKINS, RH KNIGHT 1972 Polyribosomes from peas: an improved method for their isolation in the absence of ribonuclease inhibitors. Plant Physiol 50: 581–584
4. DROUET AG, CJ HARTMANN 1979 Requirements for extraction of polyribosomes from lyophilised peel tissue of climacteric pear. Phytochemistry 18: 545–547
5. HARTMANN CJ 1962 La décarboxylation du pyruvate par les tissus de poire au cours de la maturation du fruit. Rev Gen Bot 69: 26–31
6. HARTMANN CJ 1966 Contribution à l'étude de la crise climactérique des fruits. Recherches sur quelques aspects du métabolisme respiratoire. Thesis Paris. Ann Sci Nat Bot 7: 131–214
7. HARTMANN CJ 1975 Enzymes, maturation et comportement respiratoire d'un fruit (la cerise) cueilli à intervalles réguliers après floraison et conservé à température constante. *In* Facteurs et Régulation de la Maturation des Fruits. Coll Int CNRS 238: 235–240
8. HARTMANN CJ, M BOULAY, AM MOULET 1975 Enzyme malique, maturation et besoin de froid chez la poire Passe-Crassane. CR Acad Sci Paris 281: 135–137
9. HARTMANN CJ, AG DROUET 1973 Etude par électrophorèse en gel de polyacrylamide de l'évolution enzymatique des tissus poire Passe-Crassane pendant la maturation du fruit cueilli. Physiol Plant 28: 284–290
10. HARTMANN CJ, AG DROUET 1979 Pear malic enzyme, some physical and immuno-chemical properties Rev Can Biol 38: 101–104
11. HULME AC 1937 Changes in the nitrogenous compounds of apple in the period of the climacteric rise in respiratory activity. Rep Food Invest Board Lond 1936
12. HULME AC 1954 Studies in the nitrogen metabolism of apple fruits. The climacteric rise in respiration in relation to changes in the equilibrium between protein synthesis and breakdown. J Exp Bot 5: 159–172
13. KU LL, RJ ROMANI 1970 The ribosomes of pear fruit. Their synthesis during the climacteric and the age-related compensatory response to ionizing radiation. Plant Physiol 45: 401–407
14. LEBLOND C, R ULRICH 1973 Controle de l'évolution de la maturation par divers traitements thermiques et gazeux chez la poire "Passe-Crassane." Bull Inst Int Froid 3: 69–74
15. RAMAGOPAL S, TC HSIAO 1973 Polyribosomes from maize leaves. Isolation at high pH and amino acid incorporation. Biochim Biophys Acta 299: 460–467
16. RHODES MJ 1970 The climacteric and ripening of fruits. *In* A Hulme, ed, The Biochemistry of Fruits and Their Products, Vol 1. Academic Press, London, pp 521–536
17. ROMANI RJ, K FRENCH 1977 Temperature-dependent changes in the polysomal population of senescent (ripening) pear fruit. Plant Physiol 60: 930–932
18. SACHER J 1973 Senescence and post harvest physiology. Annu Rev Plant Physiol 24: 197–310
19. STAEHELIN T, FO WETTSTEIN, H OURA, H NOLL 1964 Determination of the coding ratio based on molecular weight of messenger ribonucleic acid associated with ergosomes of different aggregate size. Nature 201: 264–270
20. STONE AB 1974 A simplified method for preparing sucrose gradients. Biochem J 137: 117–118
21. ULRICH R, A PAULIN 1954 Sur la complexité des conditions thermiques de la maturation des poires Passe-Crassane. CR Acad Agric Fr 40: 280–282
22. VASSART G, JE DUMONT, FR CANTRAINE 1971 Translational control of protein synthesis: a simulation study. Biochim Biophys Acta 247: 471–485