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ALTERATIONS IN CARBOHYDRATE METABOLISM OF γ-IRRADIATED CAVENDISH BANANA

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Key Word Index—*Musa cavendishii*; Musaceae; γ-irradiation; respiration; sugar and starch metabolism; glycolytic enzyme activities.

Abstract—γ-Irradiation of preclimacteric banana resulted in a gradual increase in fructose content, which reached a maximum in 6 days. Although the catabolism of glucose-U-^{14}C was less in irradiated banana, incorporation of label into fructose was high. Initial fructose accumulation in irradiated banana may be due to a shift in glucose utilization from the glycolytic to the pentose phosphate pathway. The ratio of resporatory CO_2 from glucose-6-^{14}C and glucose-1-^{14}C was halved in irradiated bananas indicating predominance of the pentose phosphate pathway. The radioactivity of fructose derived from glucose-6-^{14}C was almost twice that from glucose-1-^{14}C in irradiated bananas, whilst in control both fruit the labelled precursors yielded equal amounts. Studies on individual enzymes in these two pathways showed an increase in phosphorylase, phosphoglucomutase, glucose-6-phosphate dehydrogenase and fructose-6-phosphatase and a decrease in hexokinase in irradiated banana.

INTRODUCTION

THERE are a number of reports in the literature to support the idea that there is an alteration in the regular metabolic pathways of the climacteric class of fruits at the onset of ripening.[1] Tager and Biale[2] observed an increase in the activities of carboxylase and aldolase that paralleled the increase in climacteric respiration in banana. After studying the metabolism of glucose-1-phosphate, ribulose-5-phosphate, glucose-6-phosphate and hexose diphosphate in preclimacteric banana, Tager and Biale[2] suggested a shift from the pentose phosphate to EMP pathway during ripening of fruits. The increase in phosphofructokinase, the key enzyme in the glycolytic pathway, in ripening banana was closely parallel to the climacteric rise in respiration.[3] In other fruits of this class, e.g. Bartlet pears, Meynhardt *et al.*,[4] found that the pentose phosphate pathway was operative at all stages of ripening. During ripening of the mango, a four-fold increase in the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase and malic enzyme was reported by Mattoo *et al.*[5]

It is quite evident that, during the ripening of fruit, regulation of carbohydrate metabolism is important for the accumulation of sugars, organic acids and other important constituents, which contribute to the colour, texture and flavour of the fruit.

In recent years, radiation-induced delay in ripening has been reported for banana.[6–8]

[1] J. B. BIALE, *Encyclo. Plant Physiol.* **12,** 538 (1960).
[2] J. M. TAGER and J. B. BIALE, *Physiol. Plant.* **10,** 79 (1957).
[3] J. BARKER and T. SOLOMOS, *Nature, Lond.* **196,** 189 (1962).
[4] J. T. MEYENDART, R. J. ROMANI and E. C. MAXI, *South Afric. J. Agric. Sci.* **8,** 691 (1965).
[5] A. K. MATTOO, V. V. MODI and V. V. R. REDDY, *Indian J. Exptl Biol.* **5,** 111 (1968).
[6] W. E. FERGUSON, A. R. YATES, K. E. MACQUEEN and J. A. ROBB, *Food Technol. Champaign* **20,** 203 (1966).
[7] E. C. MAXIE, R. AMEZUITA, B. M. HASSAN and C. F. JOHNSON, *Proc. Am. Soc. Hort. Sci.* **92,** 235 (1968).
[8] P. THOMAS, S. D. DHARKAR and A. SREENIVASAN, *J. Food Sci.* **36,** 243 (1971).

γ-Irradiation interferes with the regular metabolism of fruit but its effects on the physiological and biochemical events of ripening is little understood. There are some reports to show that γ-irradiation interferes with carbohydrate metabolism. Massey[9] using carrot tissues has studied the effect of γ-rays on carbohydrate and organic acid metabolism. These studies have pointed out that the Embden–Meyerhof (EMP) pathway was found to be more sensitive to radiation than the pentose phosphate pathway. Recently Ussuf and Nair[10] reported that γ-irradiation of potatoes at 10 krad caused an activation in catabolism of sugar phosphate esters via the Kreb's cycle. In the present investigation we have attempted to study some distinct changes in carbohydrate metabolism which may be responsible for the delay in ripening of γ-irradiated banana.

RESULTS

One of the direct and immediate effects of γ-irradiation is an increased respiration rate, which in some cases can be sustained for several days.[11,12] This response is not given by all fruits of the climacteric class. In the case of freshly harvested bananas grown in India, Thomas *et al.*[8] has reported that maximum delay in ripening was obtained only when they were in the pre-climacteric stage. The maturity of the fruit and its physiological state (its stage in the climacteric sequence) has a profound influence on its response to radiation. The respiration studies with 70% mature pre-climacteric Cavendish bananas clearly established that the onset of the climacteric was postponed for at least a week in a 35-krad irradiated sample (Fig. 1). They did not show an upsurge in respiration as reported for

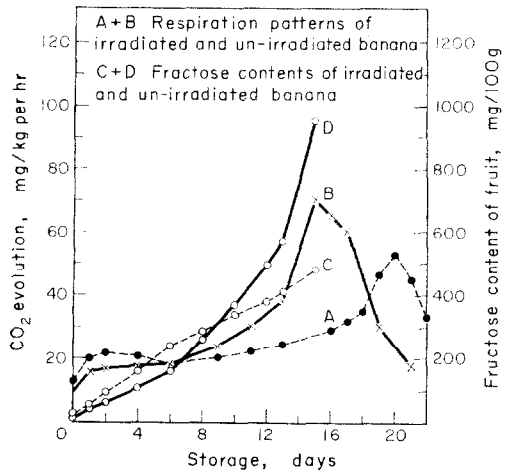

FIG. 1. EFFECT OF γ-IRRADIATION ON THE RESPIRATION AND FRUCTOSE CONTENT OF CAVENDISH BANANA

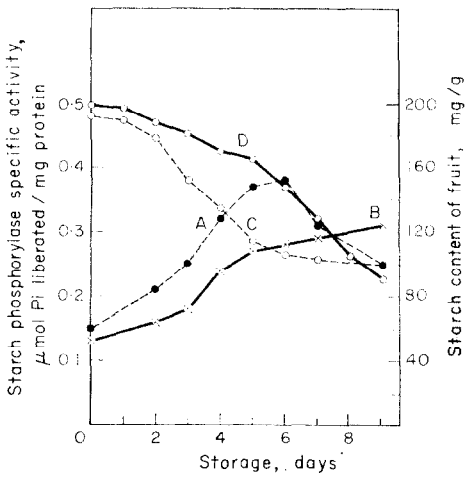

FIG. 2. INCREASE ON STARCH PHOSPHORYLASE ACTIVITY AND DECREASE IN STARCH CONTENT OF γ-IRRADIATED BANANA.

[9] L. M. MASSAY, JR., in *Preservation of Fruits and Vegetables by Radiation*, Proc. of a Panel on preservation of fruits and vegetables by radiation, p. 195, I.A.E.A., Vienna (1968).
[10] K. K. USSUF and P. M. NAIR, *J. Agric. Food Chem.* **20**, 282 (1972).
[11] R. J. ROMANI, *Adv. Food Res.* **15**, 57 (1966).
[12] L. M. MASSEY, JR., D. F. TALLMAN and Z. I. KERTEZ, *J. Food Sci.* **20**, 389 (1961).

other climacteric fruits such as Bartlet pears[13] and mangoes,[14] although at 24 hr there was a slight increase in respiration rate.

TABLE 1. UTILIZATION OF GLUCOSE-U-^{14}C IN UNIRRADIATED AND IRRADIATED BANANA

Sample	CO_2 liberated 0 days cpm	CO_2 liberated 0 days Sp. act. (cpm/mg CO_2)	CO_2 liberated 6 days cpm	CO_2 liberated 6 days Sp. act. (cpm/mg CO_2)	Incorporation into sugars (cpm × 10^{-5}) 0 day Sucrose	0 day Fructose	6 days Sucrose	6 days Fructose	Unutilized glucose in pulp 80% EtOH extra (cpm × 10^{-5}) 0 day	6 days
Unirradiated	5137 ± 89	1556 ± 11	18 008 ± 101	3199 ± 44	8·5 ± 0·10	3·2 ± 0·08	8·6 ± 0·19	9·1 ± 0·20	30·0 ± 0·23	5·5 ± 0·13
Irradiated	5448 ± 67	1238 ± 26	9327 ± 87	2618 ± 37	11·0 ± 0·12	5·2 ± 0·12	12·5 ± 0·21	15·0 ± 0·14	90·0 ± 0·20	62·0 ± 0·13

Values given are mean ±s.e.

There is a direct relationship between the respiration of the fruit and its carbohydrate content.[1] In order to understand fully the effect of γ-irradiation, a study on the carbohydrate metabolism was undertaken. A determination of reducing sugars in both control and irradiated samples at 24 hr intervals showed a high concentration of fructose in the irradiated sample within 3 days. At 6 days the fructose content was about 50% more than that of control (Fig. 1). After 9 days as the unirradiated banana started ripening, fructose content increased at a faster rate. The concentration of glucose in the irradiated sample did not show any pronounced change from controls during the initial stages of storage.

TABLE 2. HYDROLYSIS OF SUCROSE-U-^{14}C IN UNIRRADIATED AND IRRADIATED BANANA

Sample	cpm × 10^{-5} Sucrose	Glucose	Fructose
Unirradiated	7·1 ± 0·12	40·0 ± 0·21	41·0 ± 0·23
Irradiated	13·2 ± 0·14	57·0 ± 0·09	57·0 ± 0·18

Values given are mean ±s.e. 20 μCi sucrose-U-^{14}C (sp. act. 78 mCi/mmol) was injected.

The utilization of glucose-U-^{14}C was examined to find the source of fructose in irradiated banana. The 80% ethanol extract of the pulp after purification by ion exchange and PC was taken to determine the incorporation of label from glucose, into sucrose, and fructose. The distribution of label into skin and pulp tissue of the fruit showed that only 70% the label could be recovered in 80% ethanol extract. The rest of the label was distributed in pulp residue (10%), 80% ethanol extract of skin (18%) and skin residue (2%). So it was difficult to determine the exact amount of glucose utilized. However, the nature of glucose utilization could be obtained from a comparison of radioactivity in unutilized glucose in the 80% ethanol soluble fraction of irradiated and unirradiated banana. The data given in Table 1 suggest a decreased utilization of glucose in the irradiated sample. In these studies one has to consider the altered permeability of the fruit tissue as a result of γ-irradiation which might affect the uptake of glucose. Reports on the effect of irradiation on the

[13] E. C. MAXIE, N. F. SOMMER, C. MULLER and H. L. RAE, *Plant Physiol.* **41**, 437 (1966).
[14] S. D. DHARKAR, K. A. SAVAGAON, A. N. SREERANGARAJAN and A. SREENIVASAN, *J. Food Sci.* **81**, 863 (1966).

permeability of fruits and vegetables are scanty. Bourke and Massey[15] observed that in carrot tissue slices the uptake of glucose was affected only at very high doses (500 krad) of γ-rays; below 100 krad they found no effect. If this was true in the case of banana also, then the interference on glucose uptake by irradiation at 35 krad would be negligible. However, further work is necessary to clarify this point.

The radioactivity in respired CO_2 was also higher in the unirradiated sample. Nevertheless, fructose isolated from irradiated banana always had about 62·5% more radioactivity, which supported the idea fructose accumulated as a result of irradiation. The excess production of fructose can be due either to predominance of the pentose phosphate pathway or to an increase in sucrose hydrolysis. However, studies with sucrose-U-^{14}C suggested that sucrose hydrolysis was not affected by irradiation (Table 2). The alternative explanation was tested by determining the ratio of CO_2 liberated from glucose-6-^{14}C and glucose-1-^{14}C (Table 3). The ratio of specific activities of CO_2 evolved from glucose-6-^{14}C/glucose-1-^{14}C was halved in irradiated banana indicating a shift from the glycolytic to pentose phosphate pathway.[16] Additional support for the accelerated functioning of pentose phosphate pathway came from the determination of radioactivity of fructose formed from glucose-1-^{14}C and glucose-6-^{14}C in 2 hr after administration of label. In unirradiated banana the radioactivity in fructose derived from both the specifically labelled glucose molecules did not show any difference indicating that glucose utilization occurs mainly through the glycolytic pathway. But, in irradiated banana, label from glucose-6-^{14}C was almost twice that from glucose-1-^{14}C, providing evidence for activation of the pentose phosphate pathway.

TABLE 3. UTILIZATION OF GLUCOSE-6-^{14}C AND GLUCOSE-1-^{14}C BY UNIRRADIATED AND IRRADIATED BANANA

Sample	CO_2 liberated cpm		CO_2 liberated Sp. act. (cpm/mg CO_2)		$^{14}CO_2$ (G-6-^{14}C) / $^{14}CO_2$ (G-1-^{14}C)	Incorporation into fructose cpm × 10^{-5}	
	G-6-^{14}C	G-1-^{14}C	G-6-^{14}C	G-1-^{14}C		G-6-^{14}C	G-1-^{14}C
Unirradiated	5596 ± 56	9200 ± 73	2087 ± 42	2921 ± 37	0·71 ± 0·026	2·3 ± 0·17	3·4 ± 0·07
Irradiated	1260 ± 39	5707 ± 49	311 ± 27	824 ± 36	0·32 ± 0·011	4·0 ± 0·08	2·2 ± 0·04

Values given are mean ± s.e. 20 μCi of glucose-6-^{14}C (sp. act. 3·63 mCi/mmol) and glucose-1-^{14}C (sp. act. 4·2 mCi/mmol) was injected.

Effects on Enzyme Activities

Studies on the utilization of glucose-U-^{14}C indicated that irradiation has somehow hindered the catabolism of free glucose (Table 1), by affecting the phosphorylation and/or by increasing the phosphorolysis of starch. The glucose phosphate esters derived from starch would then dilute label of glucose-6-phosphate derived from free glucose. In irradiated banana there was an increase in starch phosphorylase which reached a maximum 4–5 days after irradiation. There was a decline in this activity below the level of control after 9 days as unirradiated fruits began to reach the climacteric peak (Fig. 2). Recent studies[10] have shown that in potatoes also a 10 krad γ-irradiation increased phosphorylase activity. Estimation of starch in irradiated banana showed a good correlation between decrease in starch content and increase in phosphorylase activity. The breakdown of starch occurred

[15] J. B. BOURKE and L. M. MASSEY, JR., *Radiat. Res.* **31,** 783 (1967).
[16] J. KATZ and H. G. WOOD, *J. Biol. Chem.* **235,** 2165 (1960).

at a higher rate in irradiated banana. This means that more glucose-1-phosphate was made available for catabolism.

A comparative study on the activities of three enzymes, namely, hexokinase, phosphoglucomutase, and glucose-6-phosphate dehydrogenase (Table 4) showed that there was a diminution in hexokinase activity, whilst phosphoglucomutase and glucose-6-phosphate dehydrogenase activity increased. The observed inhibition of hexokinase on irradiation caused a retardation in the initial phosphorylation and further utilization of free glucose. The increase in phosphoglucomutase facilitated the formation of glucose-6-phosphate from glucose-1-phosphate derived from the phosphorolysis of starch. Activation of G-6-P dehydrogenase adds support to the postulated shift from the glycolytic to the pentose phosphate pathway in irradiated banana. The enhanced function of the pentose phosphate pathway increased the production of fructose-6-phosphate and fructose-1,6-diphosphate, which may be hydrolysed by their respective phosphatases. Our previous studies[17] showed an activation of fructose-1,6-diphosphatase in γ-irradiated banana. This activation reached a maximum in 3 days after irradiation. Similarly a two-fold increase in fructose-6-phosphatase was also observed immediately after irradiation (Table 5). Therefore, the increase in the formation of labelled fructose in irradiated banana can be attributed to the predominance of the pentose phosphate pathway.

TABLE 4. EFFECT OF γ-IRRADIATION ON HEXOKINASE, PHOSPHOGLUCOMUTASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITIES OF BANANA

Sample	μmoles of NADPH formed/min/mg protein								
	Hexokinase			Phosphoglucomutase			G-6-P dehydrogenase		
	0 day	3 days	6 days	0 day	3 days	6 days	0 day	3 days	6 days
Unirradiated	26	140	150	150	1480	1600	24	300	250
Irradiated	23	118	125	200	1960	1890	40	370	320

The values given are mean of four independent determinations.

The activation of these enzymes was dependent on irradiation dose (Table 6). The dose which delayed the ripening for this variety was most effective in the activation of phosphoglucomutase, G-6-P dehydrogenase, phosphorylase and fructose-6-phosphatase. Higher doses caused inhibition of these activities. At 300 krad there was considerable reduction in all enzyme activities. Hexokinase activity, on the other hand showed a steady decrease as the dose was increased. Thus, this activation of enzyme activity is dose specific, which suggest that it is not due to increased extractability of enzymes as a result of irradiation. Moreover, the activation was not observed when banana tissue slices were irradiated at 35 krad. Recent studies have clearly established that γ-irradiation is capable of activating polyphenol oxidase in banana[18] and asparagine synthetase in potatoes.[19,20] Banana is known to be rich in polyphenols and tannins, which will interfere with cell-free preparation of enzymes.[21–23] In enzyme studies on such tissues, additives such as PVP are incor-

[17] K. K. SURENDRANATHAN and P. M. NAIR, *Phytochem.* **11,** 119 (1972).
[18] P. THOMAS and P. M. NAIR, *Phytochem.* **10,** 771 (1971).
[19] P. M. NAIR, *Arch. Biochem. Biophys.* **133,** 208 (1969).
[20] P. M. NAIR and A. SREENIVASAN, *Indian J. Biochem. Biophys.* **8,** 204 (1971).
[21] W. D. LOOMIS and J. BATTAILE, *Phytochem.* **5,** 423 (1966).
[22] A. C. HULME and J. D. JONES, in *Enzyme Chemistry of Phenolic Compounds* (edited by J. B. PRIDHAM), p. 97, Pergamon Press, Oxford (1963).

porated in the extraction medium to combine with tannins and polyphenols and release the enzyme in solution.[22] In a recent study by Baijal *et al.* on the isolation of enzymes from various parts of banana plant, Triton X100 was proved to be better than PVP for this purpose.[24] In our studies on the isolation of four enzymes (starch phosphorylase, hexokinase, phosphoglucomutase, and G-6-P dehydrogenase), the presence of 1% Tween 80 in the extraction medium gave better activity than a medium containing 1% PVP.

TABLE 5. EFFECT OF γ-IRRADIATION ON FRUCTOSE-6-PHOSPHATASE OF BANANA

Sample	n mol of Pi/mg protein 0 day	3 days	6 days	Sample	n mol of Pi/mg protein 0 day	3 days	6 days
Unirradiated	100	180	245	Irradiated	294	344	444

DISCUSSION

From the foregoing observations it is evident that an important metabolic event in banana after γ-irradiation is a shift from the glycolytic to the pentose phosphate pathway.

TABLE 6. EFFECT OF DIFFERENT DOSES OF γ-IRRADIATION ON HEXOKINASE, PHOSPHOGLUCOMUTASE, G-6-P DEHYDROGENASE, PHOSPHORYLASE AND FRUCTOSE-6-PHOSPHATASE

Dose (krad)	μmol of NADPH formed/min/mg protein Hexokinase 0 day	3 days	Phosphoglucomutase 0 day	3 days	G-6-P dehydrogenase 0 day	3 days	nmol of Pi liberated/mg protein Phosphorylase 0 day	3 days	F-6-Phosphatase 0 day	3 days
0	32	116	180	1195	32	250	120	160	94	198
35	25	97	265	1976	50	348	138	204	218	176
70	10	41	169	1021	30	243	130	156	122	157
100	5	14	128	535	22	89	96	112	65	94
300	2	9	44	123	15	36	40	56	40	65

The most used method of assessing the relative contribution of glycolytic and pentose phosphate pathways is to use specifically labelled glucose molecules and to determine the ratio of CO_2 formed from them.[25,26] The fact that fructose formed from glucose-6-^{14}C contains twice as much radioactivity as that from glucose-1-^{14}C (Table 3) also supported a shift towards pentose phosphate pathway in irradiated banana. Additional evidence for this shift was obtained when the activity of G-6-P dehydrogenase was studied in control and irradiated banana. Immediately after irradiation about 66% increase in this activity was observed. On the third day, the activities of control as well as irradiated samples increased, but irradiated material still showing higher activity. This higher level of activity was maintained even after 6 days in the irradiated sample, whereas in control banana there was a decline in this activity. This observation agrees well with the suggestion of Tager and Biale[2] that there is shift from the pentose phosphate to glycolytic pathway in ripening bananas. A study of G-6-P dehydrogenase in banana at different stages of ripening (Fig. 3)

[23] J. W. ANDERSON, *Phytochem.* **7**, 1973 (1968).
[24] M. BAIJAL, S. SINGH, R. N. SHUKLA and G. G. SANWAL, *Phytochem.* **11**, 929 (1972).
[25] H. G. WOOD, J. KATZ and B. R. LANDON, *Biochem. Z.* **338**, 809 (1963).
[26] E. ELA, W. CHEFURKA and J. R. ROBINSON, *J. Insect. Physiol.* **16**, 2137 (1970).

revealed that as ripening proceeded G-6-P dehydrogenase activity declined. The minimum level was obtained between 9 and 12 days when the climacteric upsurge in respiration began. As ripening was completed, the G-6-P dehydrogenase activity increased.

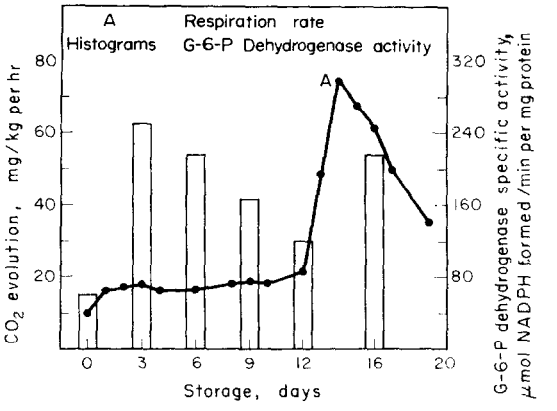

FIG. 3. GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY AT DIFFERENT STAGES OF RIPENING.

The decrease in hexokinase activity is consistent with the observed radiation-induced decrease in the metabolism of sugars reported in animals[27] as well as in plants.[9] Experiments of Bourke and Massey[15] on the effect of radiation on carrot tissue using C-1 and C-6 labelled glucose also indicated a faster metabolic rate for C-1 of glucose confirming activation of the pentose phosphate pathway. Faust *et al.*[28] have also reported that radiation can alter the main respiratory system from the EMP pathway to pentose phosphate pathway within 3 weeks of irradiation of apples. The breakdown of storage starch to glucose-1-phosphate provided enough substrate for the functioning of the pentose phosphate pathway which contributed the bulk of CO_2 liberated after irradiation. The activation of fructose-1, 6-diphosphatase[17] would check the production of the key intermediate for the glycolytic pathway, namely fructose-1,6-diphosphate. Thus the regulation of carbohydrate metabolism plays an important role in the delay in ripening of banana induced by γ-rays.

EXPERIMENTAL

Cavendish bananas (*Musa cavendishii*) used in these studies were harvested at 70% maturity as assessed by the pulp to skin ratio of a representative fruit after harvesting.[8] The respiration of the harvested banana was taken as the index of preclimacteric state. The respiration rate at this stage was between 10 and 15 mg of CO_2/hr/kg of banana. Individual fruit were separated and the cut ends were smeared with a fungicidal paste containing pentachloronitrobenzene to prevent fungal rot during storage. To avoid changes due to difference in maturity, bananas were selected from the same bunch. The fruits were irradiated on the same day of harvest after the latex flow had ceased. Irradiation was carried out in a gamma cell-220 (Atomic Energy Canada Ltd.) in air at 25° at a dose rate of 2·76 krad/min. The fruits were given 35 krad, which was the dose for delay in ripening for this variety.[8] Ferrous sulfate (Fricke's) dosimetry was employed. For storage studies, banana were kept in perforated polythene bags at 20° and 85% relative humidity.

Glucose-1-^{14}C, glucose-6-^{14}C, glucose-U-^{14}C and sucrose-U-^{14}C used in these studies were obtained from Isotope Division, BARC.

Respiration studies. Carbon dioxide was estimated by the method described by Thomas *et al.*[8] The CO_2 evolved in 2 hr at 25° was trapped as $BaCO_3$ using 0·1 N Ba $(OH)_2$ and expressed as mg CO_2 evolved per kg of banana per hr.

Separation and estimation of reducing sugars. After determining respiration, the same banana was used to isolate reducing sugars. Soluble sugars were extracted with 80% EtOH, the extract concentrated under

[27] G. HEVSEY and A. FORSBERG, *Nature, Lond.* **168**, 692 (1951).

[28] M. FAUST, B. R. CHASE and L. M. MASSEY, JR., *Proc. Am. Soc. Hort. Sci.* **90**, 25 (1967).

reduced pressure, and amino acids and organic acids separated from sugars by passage successively through Dowex 50 H^+ and Dowex-1-formate columns. The eluate from the column was concentrated, made up to a known volume and aliquots were spotted in duplicate on a Whatman No. 3 paper. The chromatogram was developed with BuOH–HOAc–H_2O (4:1:5) solvent system for 72 hr. The sugar spots were detected using aniline diphenylamine phosphate reagent. Individual sugars in the unsprayed strip were located by comparing the spots in the sprayed portions. The reducing sugars were eluted from the paper with H_2O and glucose was estimated using Nelson's method[29] and fructose was determined according to Roe's resorcinol method.[30]

Incorporation of glucose-U-^{14}*C into* CO_2*, fructose and sucrose.* 20 μCi of glucose-U-^{14}C was injected into two slots of 2 mm dia. and 15 mm depth made by a stainleess steel corkborer, equi-distant from both ends of control and irradiated banana. The respired CO_2 (2 hr) was collected in $Ba(OH)_2$ as described earlier, $BaCO_3$ was filtered, washed, dried, weighed and counted in the Beckman liquid scintillation counter using Cab-o-sil gel. Reducing sugars were isolated as described above. After chromatographic separation the paper strips corresponding to individual sugars were cut and counted for radioactivity. In order to ascertain that there was uniform distribution of labelled glucose into banana, glucose-U-^{14}C was injected and after 1 hr. transverse and longitudinal sections were made and radioactivity in each section was determined. There was uniform distribution of label from tip to stem.

*Catabolism of glucose-*1-^{14}*C; glucose-*6-^{14}*C and sucrose-U-*^{14}*C.* 20 μCi of labelled precursors were injected into control and irradiated banana. The rest of the experimental procedure was as above.

Enzyme preparation and assays

Starch phosphorylase.[31] 50 g of banana pulp were weighed and homogenized in a Waring blendor with 50 ml of 0·2 M phosphate buffer pH 6·7, containing 1% Tween-80 at 0–4°. The homogenate was filtered through a cheese cloth. The filtrate was centrifuged at 20 000 *g* in the cold. The supernatant was used as the source of enzyme, which was assayed by the procedure of McCready and Hassid.[31] Phosphorus was estimated by the method of Fiske and Subbarow[32] and protein was determined using the buiret procedure.[33]

G-6-P dehydrogenase, phosphoglucomutase and hexokinase. The activities of these three enzymes were determined at 0, 3 and 6 days after irradiation, and compared with that of the control. 50 g of banana pulp were cut into small pieces and frozen with liquid nitrogen. The frozen mass was powdered in a pestle and mortar cooled with liquid nitrogen. The enzyme was extracted from the powder with 50 ml of 0·1 M Tris buffer pH 8·0 containing 1% Tween 80. The extract was passed through a cheese cloth and centrifuged at 16 000 *g* for 25 min in the cold. To clarify the supernatant was again centrifuged at the same speed for same time. To 50 ml crude extract 15·15 g solid $(NH_4)_2SO_4$ was added and dissolved with stirring. The solution was kept in ice bath for 5 min. The precipitate formed was collected by centrifugation and dissolved in 20 ml 0·05 M Tris buffer pH 8·0. This preparation contained all three enzyme activities. Glucose-6-phosphate dehydrogenase was assayed according to the method of Gibbs.[34] Hexokinase activity was determined by estimating glucose-6-phosphate formed using glucose-6-phosphate dehydrogenase and phosphoglucomutase activity was determined by measuring glucose-6-phosphate formed by coupling with G-6-P dehydrogenase.[35] A 0·5-ml sample of the respective reaction mixture was withdrawn and added to a 3-ml cuvette containing 100 μmol of Tris buffer pH 8·0, 30 μmol of $MgSO_4$, 0·1 ml G-6-P dehydrogenase (Cal Biochem) and 1 μmol NADP. The final vol. was made up to 3 ml. The absorbance at 340 nm was measured against a reaction mixture from which NADP was omitted.

Fructose-6-phosphatase. Activity was also determined in unirradiated and irradiated banana at 0, 3 and 6 days after irradiation. Frozen banana pulp was homogenized in a precooled Waring blendor with 5× its wt of cold acetone (−30°) for 1 min at 0–4°. The slurry was filtered. This process was repeated twice with half the original amount of acetone. The powder was dried at 0–4° and stored at −30°. 4 g of the acetone powder was mixed with 20 ml of 0·1 M Tris buffer (pH 7·5) in a precooled mortar and pestle. This mixture was allowed to freeze at −30° and thawed out by grinding. During this process the temperature of the mixture was not allowed to exceed 5°. The ground mass was filtered through cheese cloth and centrifuged at 20 000 *g* for 20 min. The supernatant was used as crude enzyme preparation. For the assay of fructose-6-phosphatase activity, the reaction mixture contained 500 μmol of Tris buffer pH 7·5, 20 μmol of $MgCl_2$ 0·4 μmol of fructose-6-phosphatase and 0·5 ml of the enzyme. The final vol. was made up to 2 ml. This

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[30] J. H. ROE, J. H. EPSTAN and N. A. GOLDSTEIN, *J. Biol. Chem.* **178,** 839 (1949).
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was incubated at 37° for 20 min. The reaction was stopped by the addition of 1·5 ml 10% TCA and Pi liberated was determined.[28]

Estimation of starch. The diastase method with subsequent acid hydrolysis as outlined in AOAC[36] was followed with some modifications. The banana pulp was extracted thoroughly with 80% EtOH to remove soluble sugars and then powdered. This powder was subjected to hydrolysis by Takadiastase and an aliquot of the supernatant was hydrolysed with HCl; reducing sugar content of the filtrate was estimated.

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