Photosynthesis & Respiration in Developing Fruits. III. Variations in Photosynthetic Capacities During Color Change in Citrus [1]

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Several studies have been made which show some of the influences of photosynthesis in the green layers of fruits upon the development of that fruit (1, 2, 3, 4, 6). During the major part of growth in oranges, lemons, and avocados, chlorophyll does not limit photosynthesis at constant light intensities (5), as might be expected. However, during the period of color change in fruits it would be possible that chlorophyll, or chlorophyll containing cells might become a limiting factor and this phase of growth has not been studied previously. Citrus fruits are admirably suited for a study of photosynthetic changes during de-greening since the transition in these fruits does not necessarily coincide with the other abrupt changes frequently associated with ripening and color change in other fruits. Changes of composition during this period may be very slight. In addition, the peel structure of the citrus fruit makes it possible to obtain relatively small samples of external tissue with a minimum of damage for use in tracer studies.

Materials & Methods

As in previous work, a Liston-Becker Infrared Carbon Dioxide Analyzer was used for rate measurements in photosynthesis and respiration (5). Flowing air measurements were made with samples contained in tubular glass chambers or with a small methacrylate plastic compartment attached to the side of the fruit sample. During photosynthesis, the light source was a 150 w projector flood lamp. Light intensity was controlled by varying the distance of the lamp from the sample (8–46 cm) or by inserting papers in the light path. Measurements of light at the sample were made with a previously calibrated incident light meter. Heat absorbing glass in a bath cooled with circulating water was inserted in the light path to absorb the infrared radiation. Temperature measurements in the compartments indicated that samples did not undergo detectable heating even when no water bath was used to cool the sample chamber. On the other hand, it was found that simple cooling of the sample compartments without the heat absorbing glass system could not prevent rapid rises in sample temperatures. Samples were normally immersed in a constant temperature bath to maintain uniformity of measurements.

The water-saturated air which was passed over the samples was led directly to the CO_2 analyzer cell without prior drying. The constant amount of moisture in the air going through the cell only added slightly to the background reading. Elimination of the large volume of the drying tube allowed rapid measurement of transient effects, using lower flow rates than would otherwise be possible. The lower flow rates, in turn, increased the sensitivity of measurements.

Labeling experiments with $C^{14}O_2$ were performed with peel disks prepared as outlined below on the specific experiments. To obtain simultaneous exposure of peel samples in the light and in the dark to identical atmospheres, all the disks were mounted on wire needles in an inverted microbell jar. The samples to be maintained in the dark were attached to the lower part of the support with a black paper light baffle mounted above them. The photosynthesis samples were placed above this light baffle. The sides of the bell jar were covered with black tape. The samples were illuminated from above through the translucent bell jar base. The baffles and external tape prevented light from reaching the dark samples. A heat absorbing unit, as described above, was inserted in the light path. Following a pre-illumination period of 30 minutes, $C^{14}O_2$ was admitted into the chamber by drawing a vacuum on the system (through the bell jar side tube) and then flushing the externally generated $C^{14}O_2$ into the system through the neck of the jar. After a 30 minute exposure, the samples were inactivated in boiling ethanol, extracted, and the activities in the various fractions and alcohol soluble components determined. Extraction, chromatographic analysis, and activity counting procedures have been described (2). Chlorophyll was determined by the method of Koski (3).

Results

Changes in Photosynthesis by Intact Fruit during Loss of Chlorophyll. Measurements were made initially on three intact oranges varying in degree of loss of chlorophyll from the full green (but full-grown) through 40 % loss to 95 % or greater loss. The curves obtained for respiration and photosyn-

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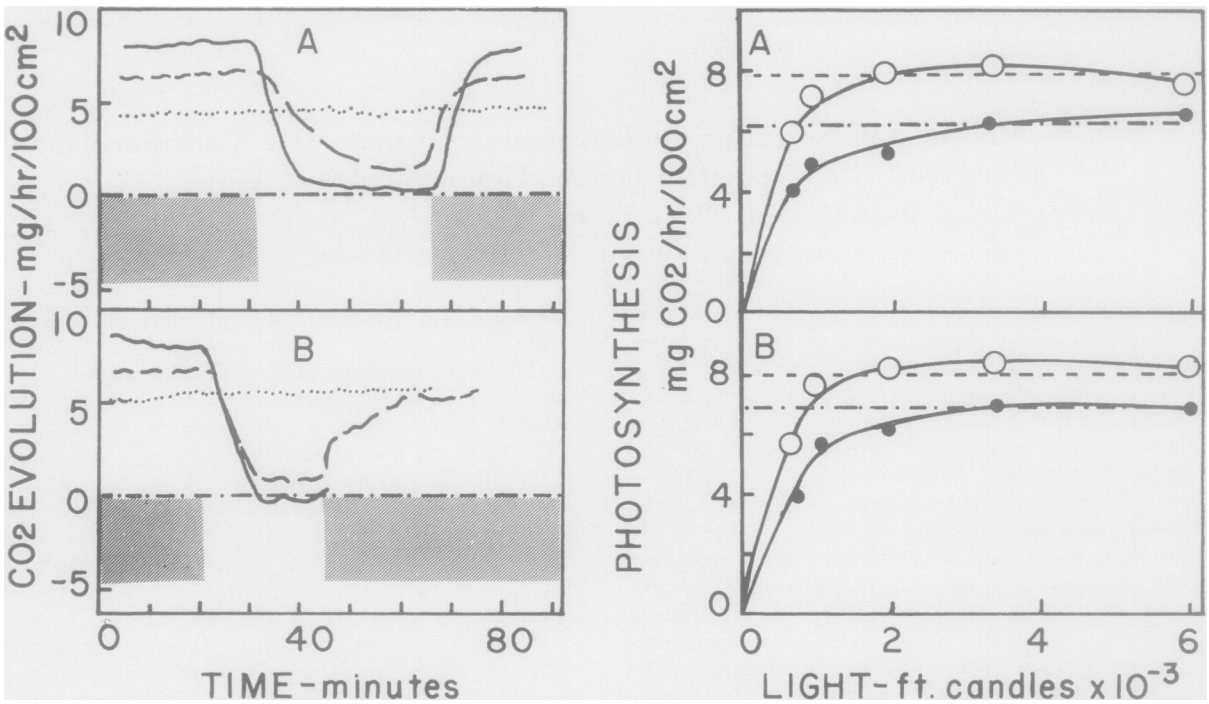

FIG. 1 (*left*). Photosynthesis and respiration of intact citrus fruits in three stages of color change. A. Oranges. Solid line, full green fruit, 25 μg chlorophyll/cm^2 surface; dashed line, part green fruit, 16 μg chlorophyll/cm^2; dotted line, full orange fruit, ca 1 μg chlorophyll/cm^2 B. Lemons. Solid line, full green, 19 μg chlorophyll/cm^2 surface; dashed line, part green, 12 μg chlorophyll/cm^2; dotted line, full yellow fruit, $<$ 1 μg chlorophyll/cm^2. The curves shown are tracings of the CO_2 analyzer recordings made with a flow compartment attached to the side of the fruit so that an area of peel 20 mm in diameter was exposed to the flowing air stream. The dashed and dotted line at zero indicates the point at which respiratory loss and photosynthetic uptake of CO_2 are equal (compensation level) and no net change occurs in the flowing air stream. Shaded bars indicate dark periods. Light at 1700 ft.-c.

FIG. 2. (*right*). Variation in photosynthesis in citrus fruits with light intensity. A. Oranges. O-full green fruit, 25 μg chlorophyll/cm^2 surface. ●-part green, 16 μg chlorophyll/cm^2. Full orange fruit showed no photosynthesis. The dashed line shows the compensation level for the full green fruit, the dashed and dotted line for the part green fruit. B. Lemons O-full green, 19 μg Chlorophyll/cm^2. ●-part green, 12 μg chlorophyll/cm^2. Compensation lines as indicated in part A.

thesis in these fruits, using a side compartment for flow measurements with the infrared analyzer, are given in figure 1 A. Similar curves are given for lemons in figure 1 B. It may be seen here that respiration decreased somewhat and that photosynthesis also decreased, roughly in proportion to the loss in chlorophyll. The full orange-colored or full yellow-colored fruits did not carry on sufficient photosynthesis to be detected by the infrared procedure but, as shown in a later section, disks from the peel of these same fruits were capable of fixing $C^{14}O_2$ nearly in proportion to the amount of residual chlorophyll in the samples. It should be noted, however, that the photosynthetic rate relation to chlorophyll only holds true within a given fruit. The lemon, despite having a substantially lower chlorophyll content at the full green stage still maintained a higher rate of photosynthesis than the orange.

Variation in Photosynthesis with Light Intensity. Lemons and oranges at three different stages of color development were tested as intact fruits to determine the optimal light levels for photosynthesis. Figure 2 illustrates the typical curves obtained for variation in photosynthesis with light intensity for oranges and lemons at two stages of color. The third stage, full orange or full yellow color with no chlorophyll, gave no detectable photosynthesis. Similar results were also obtained using intact fruits in a tubular chamber illuminated only on one side except that somewhat higher light intensities were required to attain apparent maximal photosynthesis.

In both fruits, maximal photosynthesis did no more than compensate for respiratory loss of CO_2. Increasing the light past 2000 ft-c did not stimulate CO_2 utilization significantly past the compensation level. There was an apparent difference in the photosynthetic capacity of the fruits with different chlorophyll content here but the true photosynthetic capacities may not be represented here as will be seen in other experiments.

$C^{14}O_2$ Studies and Effect of Tissue Injury upon Photosynthesis. Many of the tests for photosynthesis

Table I

Influence of State of Division upon Photosynthetic & Respiratory Rates in Orange Peel

Samples prepared as described in text. Respiration & photosynthesis were measured in flow tubes with the infrared CO_2 analyzer. Light level was about 2000 ft-c for all samples. Both respiration & photosynthesis are given as mg CO_2/hr/100 cm² of external surface (flavedo) for consistent comparisons.

Sample	Respiration mg CO_2/hr/100 cm²	Photosynthesis mg CO_2/hr/100 cm²
Intact fruit	8.4	7.6
One-eighth section (including juice sacs)	12.8	7.0
One-eighth section, peel only	8.2	7.5
One-eighth section, flavedo only	8.9	0.4
20 mm peel disk	8.9	7.9
15 mm peel disk	9.4	5.0
10 mm peel disk	9.2	2.3
10 mm flavedo disk	7.3	0.0

and respiration would be facilitated by the use of small sections of peel tissue rather than by the use of intact fruits. This is particularly true for tracer studies. Earlier experiments with young, small fruits (2) had indicated that very small sections of orange peel were capable of effective photosynthesis and relatively normal respiration. However, preliminary experiments using carefully prepared peel sections of mature fruits showed the surprising result that there was no photosynthetic fixation of $C^{14}O_2$ in flavedo samples 10 mm in diameter. This obviously did not agree with the CO_2 analyzer results for the intact fruits. Accordingly, tests were carried out to determine the effect of subdivision and injury of the peel on respiration and photosynthesis. The peel in this experiment was carefully treated to avoid injury by bending or pressure. In the preparation of peel disks, a cork borer of appropriate diameter was used to cut a circle in the peel. Then the peel around the desired section was removed with a razor. This enabled undercutting of the disk without subjecting it to bending. On some samples a portion of the albedo was left on the section, in others the albedo was completely removed by careful cutting. As illustrated in table I, a rapid loss of photosynthesis ability occurred as the peel sections became smaller than 20 mm in diameter. Complete removal of the albedo, causing injury to the lower layers of photosynthetic cells, brought on complete inhibition of photosynthesis. Although respiration tended to increase somewhat in 20 mm peel sections, if a portion of albedo was retained photosynthetic function did not appear to be significantly affected. Table II shows complete lack of photosynthesis fixation of $C^{14}O_2$ in small peel sections with albedo removed.

The effectiveness of using peel disks of adequate size with some albedo retention to prevent damage to photosynthetic cells is illustrated in figure 3, showing the respiration and photosynthesis of peel sections from the same fruit used to obtain the curves for figure 1. These samples were disks 20 mm in diameter with only a very thin layer of albedo retained. The respiratory levels are somewhat higher than for the corresponding intact surfaces but the gross apparent photosynthesis is not greatly affected.

Similarly prepared disks from oranges and lemons in varying stages of color change showed efficient photosynthetic fixation of $C^{14}O_2$ (table III). Even the samples showing full color change to orange or yellow seemed to have a sufficient number of photosynthesizing cells to accumulate a substantial activity above that of the corresponding dark samples. Dark exchange or fixation reactions also seemed to be somewhat greater in the green fruits than the orange or yellow samples, whereas the opposite effect had been noted in the extensively injured samples.

The distribution of activity in the alcohol soluble components, as shown in tables IV and V, shows that the CO_2 fixation in these samples was quite similar to that which might be expected in leaves. In the light the major activity was in sucrose. Light also had an extensive effect on the fixation of activity into the amino acid and organic acid components. There were fairly substantial quantitative differences in the labeling patterns between the orange and lemon (e.g., the higher activity in organic acids in the lemon peel) but very little difference in components found to be labeled.

Effect of Temperature upon Photosynthesis and Respiration. Disks from a green fruit were prepared as above for determination of respiration and

Table II

Photosynthetic & Dark Fixation of $C^{14}O_2$ in Small Flavedo Disks Showing Failure of Photosynthesis Due to Injury

Orange peel disks 10 mm in diameter with albedo removed were prepared as outlined in the text. Cutting injuries on periphery & back of the disk inhibit photosynthesis completely. All samples were exposed simultaneously in a container (procedure given in text) to an atmosphere of air enriched with $C^{14}O_2$ from 2 mg $BaCO_3$ (120 μc/mg). Total volume of the system was about 700 ml. Figures given represent only the alcohol soluble activity. Alcohol-insoluble material contained activity roughly in proportion to the soluble extracts.

	C^{14} activity (cpm/mg fr wt) 10 min light	10 min dark
Experiment I		
Full green fruit	13.8	14.7
Intermediate green fruit	23.1	29.2
Orange fruit	35.2	31.2
Experiment II		
Full green fruit	14.3	12.8
Intermediate green fruit	26.5	28.4
Orange fruit	30.4	28.2

Table III

Light & Dark Fixation of $C^{14}O_2$ in Citrus Peel

Peel disks, 17 mm diam., with part of albedo retained were prepared as described in the text. Chlorophyll contents for orange & lemon peel as in figure 1 & 2, respectively. Conditions were as described for table I & in text. Samples were pre-exposed to light (2000 ft-c) for 30 minutes before admitting $C^{14}O_2$ to the chambers for a 30 minute exposure. Alcohol-soluble activity was obtained by extracting the entire disk, both flavedo & albedo, & then the albedo tissue was separated from the flavedo for separate measurements of activity in alcohol-insoluble residues.

Sample	C^{14} Activities					
	Alcohol-soluble (total cpm × 10^{-5})		Alcohol-insoluble (cpm/mg dry wt)			
			Flavedo		Albedo	
	Light	Dark	Light	Dark	Light	Dark
Orange						
Full green	106.0	6.39	1440	72	67	16
Intermediate	71.7	4.66	975	115	59	40
Orange	29.1	2.60	630	28	22	22
Lemon						
Green	101.4	5.34	2970	59	145	118
Intermediate	53.1	6.44	2530	95	104	24
Yellow	7.27	4.23	211	76	16.2	24

Table IV

Distribution of Activity in Alcohol-soluble Components of Orange Peel after Light or Dark Fixation of $C^{14}O_2$

Compound	Activities*					
	Light			Dark		
	Orange	Intermediate	Green	Orange	Intermediate	Green
Sucrose	12,400	36,900	60,800	...	...	...
Glucose + glycine**	150	1,400	4,200	...	...	...
Serine	62	292	681	34	154	134
Alanine	240	620	2,520	29	70	63
Glutamic acid	100	360	1,020	25	54	69
Aspartic acid	350	950	n.d.	12	36	30
Asparagine***	n.d.	n.d.	n.d.	108	138	113
Malic acid	85	362	725	103	231	200
Succinic acid	142	77	730	32	130	94
Fumaric acid	n.d.	n.d.	n.d.	64	230	207

* All activities given as cpm determined on paper chromatograms. Notation n.d. indicates activity was not determined.

** Glycine co-chromatograms with glucose and was not measured separately except in a few isolated cases which indicated that its activity was generally very low.

*** Asparagine co-chromatograms with sucrose so it was obscured in the photosynthetic samples.

Table V

*Distribution of Activity in Alcohol-soluble Components of Lemon Peel after Light or Dark Fixation of $C^{14}O_2$**

Compound	Activities					
	Dark			Light		
	Yellow	Intermediate	Green	Yellow	Intermediate	Green
Sucrose	1,900	30,500	55,300	...	...	...
Glucose + glycine	530	3,540	7,800	...	...	...
Serine	280	1,060	825	297	232	103
Alanine	265	3,300	3,760	134	115	69
Glutamic acid	260	1,075	785	168	154	103
Aspartic acid	n.d.	n.d.	n.d.	171	195	219
Asparagine	n.d.	n.d.	n.d.	86	39	69
Malic acid	157	475	650	171	195	219
Succinic acid	43	478	1,200	378	503	485
Fumaric acid	370	1,440	2,370	433	619	725

* See Table IV for explanatory notes.

Table VI

Effect of Temperature on Photosynthesis & Respiration in Citrus Peel & Comparison with Photosynthesis of Leaf Tissue.

Sample	Temp °	Light ft-c	CO_2 evolution mg CO_2/hr/100 cm²		Apparent photosynthesis*
			Dark	Light	
Orange peel disk	11°–12°	1700	+3.2	−1.0	4.2
		6000	+3.2	−1.0	4.2
	20°	1700	+9.9	+1.4	8.5
		6000	+10.0	+0.2	9.8
	30°	1700	+18.1	+8.0	10.1
		6000	+18.8	+0.6	18.2
Citrus leaf disk	20°	1700	+4.4	−8.3	12.7

* Apparent photosynthesis is the difference between CO_2 evolution in the dark and that in the light.

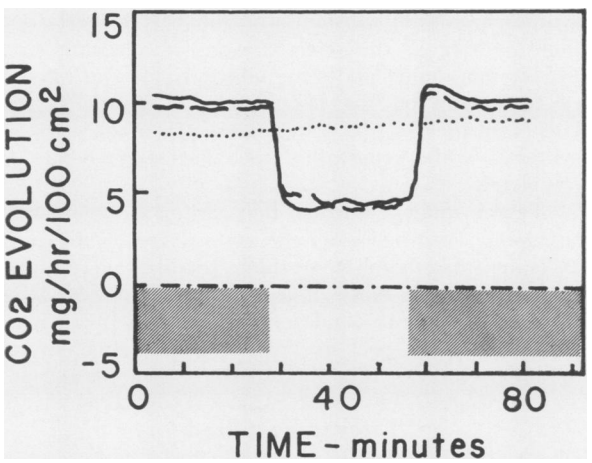

FIG. 3. Photosynthesis and respiration of peel sections from oranges. Peel disks 20 mm in diameter of the fruits in figure 1A were used. Solid line, full green fruit; dashed line, part green; dotted line, full orange fruit.

photosynthesis at three different temperatures. The first measurements were made in a water bath at 20° then the temperature of the bath was lowered to 12° and raised to 30°. A final determination of rates was made at 20° once more to determine whether the sample might have been affected significantly by the intervening time and treatments. Little difference was found between the first and last measurement in any of the samples tested. Table VI shows the data obtained for a normal temperature variation series. Respiration rates were affected strongly by the temperature changes but it was also found that photosynthesis varied almost as greatly as the respiration. Gross apparent photosynthesis at elevated temperature and 1700 ft-c was greater than the limiting photosynthesis in any of the previously tested samples. With the higher light intensity of 6000 ft-c, photosynthesis was brought once more almost to the point of equality with respiration. Increased light made no significant difference for the cooled sample but, as usual, allowed the 20° sample to reach a compensation level.

It is also of interest to compare the photosynthetic rate of the fruit surface with that of a citrus leaf surface. Although the photosynthetic rate of a single leaf surface is 50 % greater than that of the fruit surface at 20° and 1700 ft-c, increasing temperature and light upon the fruit allows its apparent photosynthetic rate to exceed that of the leaf.

Discussion

The data show, as would be expected, that photosynthesis suffers a substantial decrease in citrus fruits during the period of color change in which chlorophyll is lost and the orange or yellow colors became prominent. However, although photosynthetic rates appear to be correlated with chlorophyll content, the photosynthetic capacities do not necessarily decrease in proportion to the loss in chlorophyll. Instead, under normal conditions, the photosynthetic capacity seems to be limited much more by the respiration of the fruit than by factors concerned with the chlorophyll content. As shown by the curves for variation of photosynthesis with light intensity, the rates for fruit with different chlorophyll content appear to be roughly proportional to chlorophyll at low light intensities but at the higher light intensities the CO_2 available from respiration appears to be the limiting factor. The temperature variation studies indicate that, at higher light intensities, the fruit may be capable of a great deal more photosynthesis than they actually show under normal conditions. Thus, it appears certain that maximal photosynthesis is limited by some factor other than the primary light reaction which would be dependent on the chlorophyll concentration. It could either be the enzymes and coenzymes of the dark reactions or limitations of diffusion of atmospheric CO_2 through the thick epidermal layers of the fruit. Experiments with other fruits (unpublished) indicate that a diffusional barrier may be partially limiting in photosynthesis in the fruit.

Despite the seeming low uptake of CO_2 as measured by the CO_2 analyzer, the tracer studies indicate that exchange is rapid enough to enable a substantial labeling of photosynthetic products. The total amount of activity found in the photosynthesizing peel disks is actually quite comparable with that which might be found in a leaf disk of similar size photosynthesizing under similar conditions. Thus, although it would appear that the endogenously formed CO_2 should be preferentially utilized in photosynthesis by this tissue, the utilization of external CO_2 appears to be quite efficient, at least in the disks. The extent of exchange in mature, intact fruits remains to be determined although studies with small fruits, where respiration is a larger factor, indicate fixation may be efficient in the intact fruit as well (*2*).

An interesting anomaly which appeared in this work was the effect upon photosynthesis of division of the peel into smaller units. While flavedo sections from small fruits, in earlier experiments (*2*), had shown rather efficient photosynthesis even when as small as 2 to 5 mm in diameter, the present results, with mature fruits, demonstrated that severe inhibition of photosynthesis occurred when full peel sections were prepared smaller than 15 to 20 mm in diameter or when any attempt was made to remove the albedo layers completely from the photosynthetic flavedo layers. It may be that the difference between the small and the large fruits is due to the oil glands in the peel. In the older fruit, an incision into the peel results in a saturation of peel with oil from the injured storage gland areas to a distance of several millimeters around the cut. Such an effect is not so apparent in the small fruits with very small oil glands and cells. Additionally, closer packing of the cells of the smaller fruit tissues and higher water content probably tends to resist the movement of the oils. The complete loss of photosynthesis when the albedo tissue is removed may be due to the great number of oil glands and photosynthetic cells ruptured. The albedo tissue may also act as an aid in preventing extensive damage to the photosynthetic cells by absorbing a substantial amount of oil which would otherwise be spread through the flavedo. Surprisingly, despite its drastic effect upon photosynthesis, injury does not seem to alter respiration greatly, at least in quantitative aspects of CO_2 evolution. There is some evidence, from tracer experiments, that exchange of CO_2 in the dark reactions may be affected to some degree. Reasons for the observed divergency of inhibition in photosynthetic and respiratory systems cannot be advanced at this time.

Summary

The variations in photosynthesis and respiration of oranges and lemons during the period of transition from green fruit to orange or yellow have been studied. Intact fruit as well as peel disks were used. Changes in photosynthetic rates at low light intensities were found roughly proportioned to the chlorophyll in both lemons and oranges but photosynthetic capacities appeared to be limited more by factors which may be associated with carbon dioxide diffusion than by chlorophyll content. Photosynthetic capacities coincided with the respiratory compensation level in full green samples and in samples which had lost up to 40 % of the original chlorophyll. The equality of photosynthetic capacity and respiratory level seemed to be retained even when large changes were induced by temperature changes.

Experiments with peel disks showed that photosynthesis was not substantially impaired in peel sections as long as the sections were of adequate size (15–20 mm diam) and some albedo tissue was allowed to remain on the photosynthetic flavedo layer. Use of smaller sections could seriously inhibit photosynthesis while removal of the albedo could halt it completely.

Tracer studies indicated that carbon dioxide could be rapidly absorbed from the atmosphere by the peel disks and that the photosynthetic products were quite comparable with those of leaves.

Under selected conditions, the gross photosynthesis of orange peel may be roughly equivalent to the photosynthesis of a single surface of a citrus leaf.

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