(iv) *Effect of Storage at Low Temperature.*—Slices of tissue from matched samples of mature-green tomato fruits (cv. Tiny Tim) which had been stored at 0°C for 0, 2, 5, 8, and 12 days were infiltrated with ^{32}P and the amount of esterification after 21 hr was determined at 0°C and at 20°C. The amount of esterification at 0°C was considered to be an indication of the rate at which esterification was proceeding immediately prior to removal of the fruits from chilling conditions. The capacity for phosphorylation retained by the tissue after transfer to a ripening temperature was indicated by the amount of esterification at 20°C. For comparison, disks 5 mm in diameter were taken at weekly intervals from the leaves of a cabbage (cv. Enfield Market) during storage at 0°C for 5 weeks. Duplicate samples of disks were incubated in the presence of ^{32}P for 0·5 hr at 20°C.

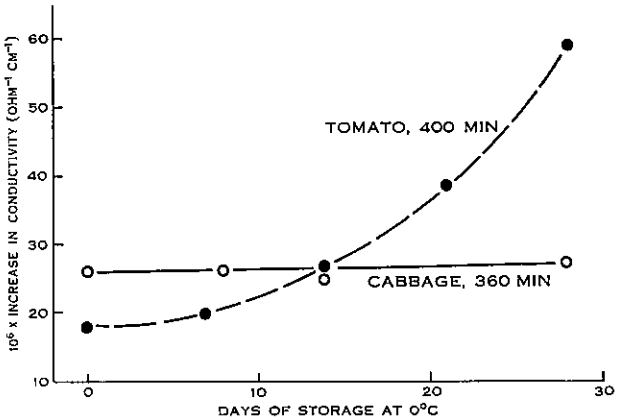

Fig. 1.—Effect of storage at 0°C on rate of leakage of electrolytes from tomato and cabbage tissue, measured as an increase in conductivity of bathing solution after periods of 400 and 360 min, respectively.

III. Results

(a) *Cell Membrane Permeability of Tomato and Cabbage Tissue*

There was a continuous increase in cell membrane permeability of mature-green tomato fruit tissue with increase in the duration of exposure to 0°C (Fig. 1). This increase in permeability was small during the first few days of chilling but became progressively more rapid with further exposure. After 4 weeks at 0°C cell membrane permeability was about three times that observed in unchilled tissue. As indicated in Figure 1, no appreciable change occurred in the permeability of the cell membrane of cabbage leaf tissue even after 4 weeks of storage at 0°C.

(b) *Phosphorylative Capacity of Tomato and Cabbage Tissue*

(i) *Effect of Incubation Temperature.*—Figure 2 illustrates the linear increase in rate of phosphate esterification with increase in incubation temperature for both tissues.

(ii) *Effect of Storage at Low Temperature.*—Exposure of mature-green tomato fruit to 0°C quickly initiated a steep decline in the capacity of the tissue for phosphorylation at 20°C (Fig. 3). This decline continued as chilling was prolonged, until after exposure to 0°C for 12 days only about one-third of the original capacity remained. Chilling also brought about a slower but significant decrease in the rate of phosphorylation at 0°C. After 12 days at this temperature the activity was only about one-half of that shown by the fruit prior to chilling. On the other hand, there was a continuous increase in the capacity of cabbage leaf tissue for phosphorylation at 20°C, and after 5 weeks of storage at 0°C this capacity was 50% greater than in unchilled tissue.

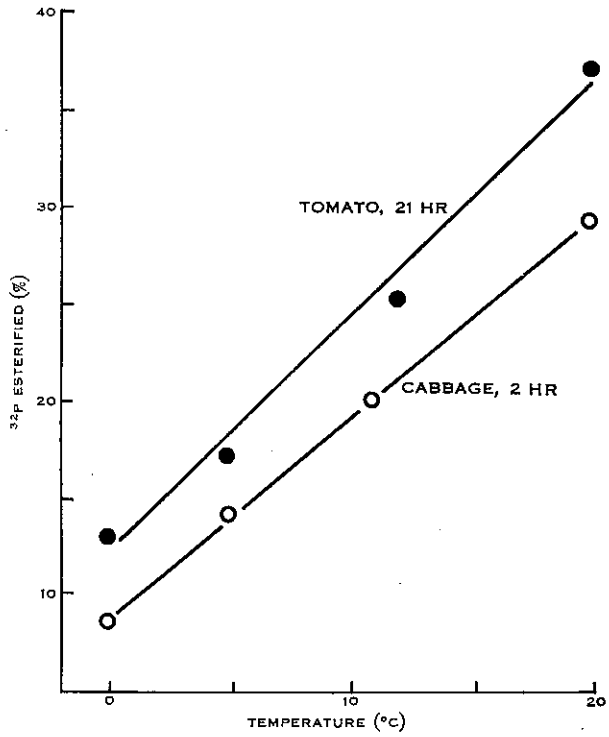

Fig. 2.—Influence of temperature on rate of ^{32}P esterification. Tomato tissue incubated for 21 hr prior to assay, cabbage tissue for 2 hr.

IV. Discussion

An increase in cell membrane permeability has been shown to occur when mature-green tomato fruits undergo relatively brief chilling treatments, but the cell membrane permeability of cabbage leaf tissue was unaffected by much longer chilling treatments. Chilling has previously been shown to elicit similar rapid responses in two other tissues which are injured by chilling, viz. mandarin endocarp (Pantanelli 1919) and sweet potato root (Lieberman *et al.* 1959). The rapidity of this response, coupled with the absence of an effect in the chilling-resistant cabbage leaf tissue,

suggests that this increase in permeability may be related to the process most directly affected by chilling.

Figure 2 shows that there is no alteration at or below the threshold temperature for chilling injury in the slope of the straight-line relationship between esterification rate of ^{32}P and incubation temperature. Thus it appears that temperatures in the chilling range do not exert any unusual influence on the rate of phosphorylation in freshly harvested mature-green tomato fruits. Moreover, the lack of any significant difference between the slopes of the straight-line relationships for tomato fruit and cabbage leaf suggests that injury does not arise from a simple lowering of the rate of phosphorylation at chilling temperatures.

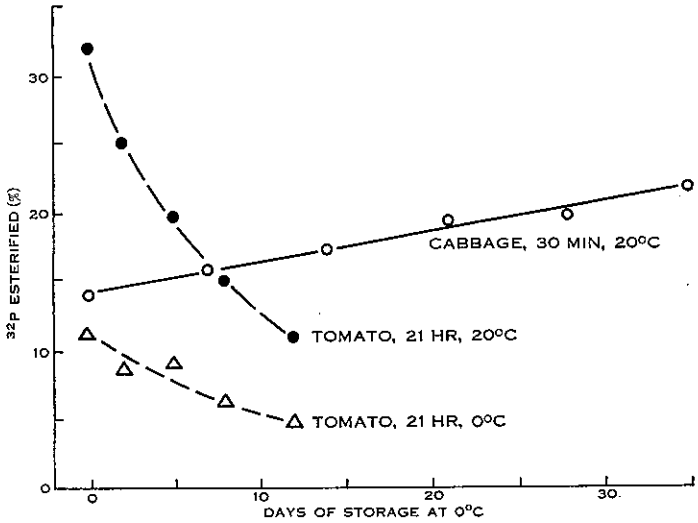

Fig. 3.—Effect of storage at 0°C on capacity of tomato and cabbage tissue for esterification of ^{32}P. Tomato tissue incubated for 21 hr at temperatures indicated prior to assay, cabbage tissue for 30 min.

However, the initial rate of phosphorylation in freshly harvested fruits placed at 0°C is not maintained, since exposure to this temperature quickly initiates a steep decline in the phosphorylative capacity of the tissue. Lieberman *et al.* (1959) observed that a decline in oxidative and phosphorylative activity commenced in sweet potato only after the roots had been held at 7·5°C (an injurious temperature) for about 4 weeks. They suggested that the apparent delay in response to chilling might be due to a masking effect of the injury sustained by the mitochondria in the normal isolation procedure. With tomato fruit tissue slices, on the other hand, the effect on phosphorylative capacity is immediate and even more marked than the effect on cell membrane permeability. This fact, together with the observation that chilling of cabbage leaf tissue results in an increase, rather than a decrease, in its phosphorylative capacity, suggests that the process of phosphorylation may be connected with that process which is primarily affected by chilling.

The progressive decline on chilling in the capacity of mature-green tomato fruit tissue for phosphorylation at the chilling temperature may lead to a shortage of energy needed for the maintenance of cellular organization. This would eventually lead to the symptoms that are observed after prolonged chilling in cases where fruits are not transferred to warmer conditions for ripening, namely, a dull, greyish appearance of the fruit, together with a loss of resistance to disease. Transfer to ripening conditions, even after much shorter periods of chilling, is followed by rapid development of fungal rot and by slow, blotchy, incomplete ripening. The more rapid development of injury symptoms following transfer to ripening conditions may perhaps be explained in the following manner. Lewis (1956) has shown that respiration rates of mature-green tomatoes following chilling are as high as, or higher than, those existing prior to chilling. While the respiratory process, and perhaps other catabolic processes, are taking place at the higher temperature at rates comparable with those obtaining before the fruit was chilled, the capacity for phosphorylation has been reduced by the chilling treatment. Because of the resultant energy deficit, a net breakdown of complex cellular components such as proteins occurs, with a consequent loss of cellular organization. This would give rise to the observed increase in cell membrane permeability, and to a lowering of the resistance of the tissue to fungal decay. The energy shortage would also prevent the normal occurrence of synthetic reactions, such as lycopene formation, which contribute to the ripening process, and which have been shown by Marks, Bernlohr, and Varner (1957) to require energy from oxidative phosphorylation. Thus the two most important symptoms of chilling injury in mature-green tomato fruits may perhaps be explained on the basis of an energy deficit arising from a chilling-induced reduction in the phosphorylative capacity of the tissue.

V. Acknowledgments

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