THE DISTRIBUTION OF ACID PHOSPHATASES DURING DEVELOPMENT OF THE FRUIT OF COCOS NUCIFERA [1]

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THE DISTRIBUTION and activity of acid phosphatases during the development and maturation of the fruit of *Cocos nucifera* L. have been investigated in connection with studies in this laboratory on the isolation and culture of the cellular components of the endosperm. During the course of its development coconut endosperm develops first as a syncytium in which the nuclei are suspended in a liquid cytoplasm and later into a cellular storage tissue. To learn something of the factors which are associated with this unique pattern of organization, and also those governing the less spectacular changes in other tissues of the developing fruit and embryo, a series of correlated investigations were undertaken. A preliminary survey of the endogenous oxygen uptake of tissues and embryos of the coconut is reported elsewhere (Cutter et al., 1952). Histochemical studies on the cellular distribution of phosphatases in *Cocos* will be presented at a later date. Sadasivan (1951) has demonstrated the presence of acid phosphatases in the endosperm and embryo of the coconut, but no extensive quantitative studies were carried out. His results were apparently based upon the enzymes extracted from a single coconut of unknown age, but apparently comparable to stage 7 of our developmental series.

EXPERIMENTAL PROCEDURE.—Colorimetric procedures were employed for the estimation of inorganic phosphate and acid phosphatase activity (Wilson and Cutter, 1952). This method utilizes the stable yellow color which is developed when an excess of molybdate is added to an acidified solution of ortho-phosphate and vanadate, and involves the determination of residual inorganic phosphate present in the tissues. After incubation with sodium glycerophosphate, inorganic phosphate is again determined. Acid phosphatase activity is calculated from the difference between total inorganic phosphate after incubation with substrate and the residual phosphate of the tissues.

Tissue homogenates were prepared volumetrically by suspending 10 g. of freshly cut tissue in 40 ml. mineral free distilled water and blending in a Waring blendor for 10 min. or until a smooth homogenate was obtained. The coconut milk was used undiluted. In calculating the results presented in fig. 1, corrections were made for the dilution of the homogenates. Tests were carried out in duplicate on 0.5 ml. of homogenate or milk from freshly opened coconuts, using 4.5 ml. of 0.5 per cent sodium glycerophosphate (Eastman 52 per cent α 48 per cent β) as substrate. The *p*H of coconut milk and homogenates was approximately 5, and since this is a suitable *p*H for the activity of acid phosphatases, stock solutions of sodium glycerophosphate were routinely adjusted to this acidity. Incubation was carried out in a water bath at 38°C. for 2 hr. Procedures for the preparation and use of reagents, standard curve and calculation of results have been described elsewhere (Wilson and Cutter, 1952).

Determinations were carried out on 88 coconuts providing a representative series of developmental stages. These were divided into 8 groups with size of the intact drupe and degree of maturation of the endosperm as criteria for estimating the developmental age. The changes which characterize the fruit during development and maturation have been described in a previous paper (Cutter et al., 1952). Fruits were collected at various times and locations in the vicinity of Miami and Key Largo, Florida; they were stored at 4°C., and were tested within 1–4 weeks after collection. Any fruits showing contamination by microorganisms were discarded.

RESULTS AND DISCUSSION.—A graphic comparison of acid phosphatase activity and inorganic phosphate concentration through the various developmental stages is given in fig. 1. Phosphatase activity is expressed in terms of the amount of phosphate liberated enzymatically under the condition of the test (dark bars). In the youngest stages (1–3) the milk, which at this time is essentially a liquid syncytium with suspended free nuclei, is characterized by exceedingly low levels of inorganic phosphate and acid phosphatase activity. During the period represented by the first three stages, although the size of the drupe increases more than threefold, the quantity of milk increases from one to several hundred milliliters, and the number of nuclei increases markedly, the acid phosphatase activity remains consistently low. The nucellus, which at this time lies in direct contact with the milk, exhibits marked enzymatic activity and also possesses a large amount of inorganic phosphate. During stage 4 the nuclei have migrated from the milk to the periphery of the embryo sac, and the greatest phosphatase activity of the endosperm can be demonstrated in this nucleated layer adjacent to and in contact with the nucellus. Concurrent with the cellular organization (stage 5), maturation (stage 6), and hardening of the solid endosperm (stage 7), is a reduction of phosphatase activity and the milk, which is now virtually devoid of nuclei, is again reduced to low levels of enzymatic

[1] Received for publication June 28, 1951.
This investigation was aided in part by a grant from the Jane Coffin Childs Memorial Fund for Medical Research and by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

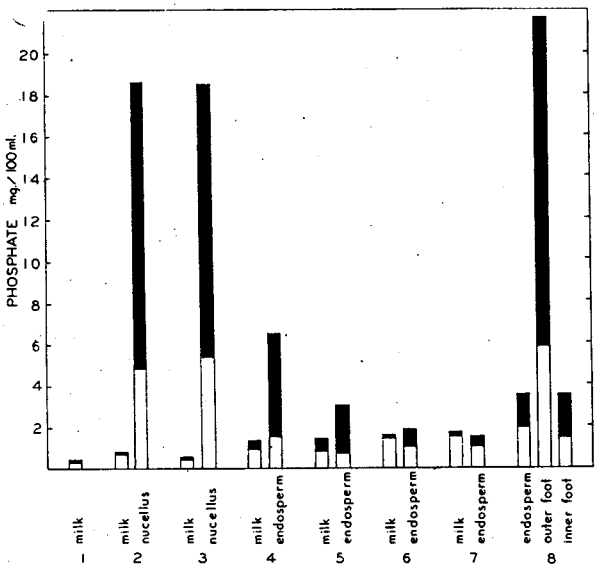

Fig. 1. Relative concentration of residual inorganic phosphate (light bars) and phosphate liberated enzymatically during 2 hr. at 38°C. (dark bars) in various tissues of *Cocos nucifera*. Concentrations are given in mg. phosphate/100 ml. of milk or 100 g. of tissue. Stages 1–8 represent average values for progressively older developmental stages. The numbers of coconuts tested in each stage are: stage 1, 5; stage 2, milk 22, nucellus 1; stage 3, milk 17, nucellus 3; stage 4, milk 5, endosperm 4; stage 5, milk 13, endosperm 13; stage 6, milk 17, endosperm 1; stage 7, milk 7, endosperm 7; stage 8, endosperm 2, outer foot 2, inner foot 2.

activity comparable to the pre-cellular stages. Since the amount of milk decreases somewhat during stages 6 and 7, either through evaporation or absorption by the cellular endosperm, the increase in inorganic phosphate may merely represent the effects of concentration.

With the development of cellular endosperm in stages 5 and 6, the nucellus atrophies, becomes non-functional and phosphatase activity can no longer be demonstrated. In mature germinating coconuts the milk has been completely resorbed and the cavity in the cellular endosperm is occupied by the devolping haustorium or foot of the embryo (stage 8). Here the endosperm, which is in process of digestion by the outer layer of the haustorium, exhibits increased acid phosphatase activity. This may represent a diffusion of enzyme from the rugose outer layer of the foot which is highly active at this time. It is interesting that this high phosphatase activity is closely localized in the area adjacent to the endosperm, and that the inner parenchymatous portion of the absorbing organ does not show great enzymatic capacity. Giri and Sreenivasan (1938) have pointed out a similar situation during the germination of cereals. Histochemical studies of the distribution of acid phosphatases within the embryo during progressive stages of its development are now in progress.

The sites of high phosphatase activity in the developing coconut show an interesting correlation with those tissues in which high endogenous rates of oxygen consumption have been demonstrated (Cutter et al., 1952). The meristematic tissues of the nucellus and the enzyme secreting regions of the foot show the greatest phosphatase activity, whereas in this respect the endosperm, even during the stages where cellular organization is proceeding, is relatively inactive. However, at that period when the nuclear-cytoplasmic ratio of the developing endosperm is highest (stage 4) there appears to be a significant increase in phosphatase activity. As has been pointed out elsewhere (Cutter et al., 1952) the expected increase in rate of endogenous aerobic respiration at this stage could not be demonstrated.

SUMMARY

The distribution and activity of acid phosphatases and inorganic phosphate have been surveyed throughout the development of the coconut. The highest acid phosphatase activity is encountered in the meristematic nucellus and the outer layers of the haustorium of the embryo. This activity is associated with those tissues which show the highest concentration of inorganic phosphate. Throughout its development the endosperm shows relatively low concentrations of inorganic phosphate and acid phosphatase activity, but the onset of cellular differentiation in this tissue is accompanied by a rise in phosphatase activity.

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