Technical Papers

The Isolation of Living Nuclei from the Endosperm of *Cocos nucifera*[1]

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In recent years a number of techniques have been developed for the isolation of large volumes of nuclei from various animal tissues (*1, 2*). In contrast, the isolation of appreciable volumes of plant nuclei is complicated by the presence of rigid cell walls and by the fact that the ratio of nuclei to cytoplasmic materials in plants is usually lower than in animal organs.

In the coconut fruit (*Cocos nucifera* L.) the endosperm develops into a cellular tissue from a syncytium in which free nuclei are suspended in a liquid cytoplasm. Prior to cellular differentiation a stage occurs at which a large number of nuclei are closely aggregated at the walls of the embryo sac. Since these nuclei are large and only loosely embedded in a gelatinous, cytoplasmic matrix the endosperm at this stage is ideal for the isolation of large volumes of living, undamaged nuclei. The fact that coconuts are readily available on a year-round basis, and can be maintained for considerable periods in a living and sterile condition under refrigeration, enhances the usefulness of this material for studies on nuclear structure, constitution, and culture.

During the early development of the embryo sac the endosperm nuclei are suspended in the liquid cytoplasm or milk, but as the coconut matures these nuclei migrate to the periphery of the embryo sac and there undergo rapid multiplication. At the same time cytoplasmic materials coalesce around these nuclei, and presently this hyaline gelatinous mass is subdivided by cell walls. This tissue subsequently matures into the familiar hard coconut meat. The various developmental stages in the maturation of this endosperm have been described and illustrated elsewhere (*3*).

In those early stages where the nuclei are suspended in the coconut milk it is possible to harvest them by differential centrifugation and washing. However, much greater yields of nuclei are obtained from the "jelly" at the periphery of the embryo sac immediately prior to the delimitation of secondary cell walls. At these stages, the nuclei must be separated from considerable amounts of ergastic materials of an oily and crystalline nature, as well as from fragments of cell wall material. The separation described here is based upon the principle that the nuclei are the largest particles in the homogenate after preliminary filtering has removed cell wall materials, and they may be retained on a filter pad while the finer cytoplasmic materials, oils, and fats are washed away. The preparation should be carried out at low temperatures and with precooled solutions in order to prevent enzymatic decomposition.

Coconuts of the appropriate age are split and the milk is removed. The developing jellylike endosperm is gently scraped out with a spatula and suspended in 100 ml cold 4% aqueous sucrose solution in a Waring blendor. Cracked ice is added, and the mixture is blended for 5 min at 110 v. A pad composed of three layers of short-fiber, bleached, absorbent cotton wool (Johnson & Johnson, Middlesex brand) is sandwiched between single layers of 15-denier, 50-gauge nylon mesh and fitted into a Büchner funnel. The edges of the pad are crimped to the rim of the funnel to prevent seepage, and the pad is then saturated with cold sucrose solution. The endosperm homogenate is strained through 4 layers of washed cheesecloth, thus removing the coarse particles and cell wall debris, and diluted to approximately 500 ml with cold sucrose solution. This diluted homogenate is slowly poured through the cotton filter pad without suction. The filtrate is collected and repassed through the pad several times in order to entrap all the nuclei within the cotton fibers. After several passages through the pad the filtrate, which now contains only fine cytoplasmic particles, is discarded.

The pad is then washed by allowing 1000 ml of cold sucrose solution to percolate slowly through the fibers without suction. This washing removes any fine particles still enmeshed in the cotton fibers or adhering to the nuclei, and the filtrate is again discarded. When the pad has drained, another 1000 ml of cold sucrose solution is poured over it and drawn through with strong suction. As the pad drains it is pressed gently to force through all the solution. The majority of nuclei trapped in the cotton fibers will be brought down in this fraction. If this procedure is followed carefully the nuclei will be virtually free of cytoplasmic debris, and any small particles may be removed by slow speed centrifugation. The nuclei are finally concentrated by centrifuging in 50-ml tubes for 10 min at 2040 rpm.

As an example of the yield of nuclei obtainable by this method, 0.130 g undamaged nuclei may be isolated from a single coconut with a gross weight of 2000 g, containing 275 ml milk and 42.8 g gelatinous endosperm.

The nuclei prepared by this technique are living, unplasmolyzed, and undamaged and are in excellent condition for enzymatic and respiratory studies. By appropriate modifications the entire process may be carried out under sterile conditions, and the nuclei subsequently maintained in sterile culture. Prelimi-

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nary experiments also indicate that nuclei may be isolated by the same procedure from the young endosperm of corn and from the storage parenchyma of watermelon fruits. Further aspects of these problems will be published later.

References

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Delayed Speech Feedback as a Test for Auditory Malingering

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Investigators in the fields of speech pathology and communication have recently become interested in the effects of delayed feedback or side-tone delay on speech. Lee has noted that

> in order to produce delayed speech feedback, it is necessary to return the speaker's speech to his own ears approximately one-quarter second after he has spoken. This is best accomplished by means of a magnetic tape recording and reproducing machine which has independent circuits and magnets. A pair of earphones should be used to experience the effect prominently since they deliver delayed speech and exclude the normal air-borne undelayed sound which provides the normal monitoring signal (*1*).

Changes that occur in the speech pattern as a result of delayed feedback have been mentioned by some investigators as being an alteration of the normal melody pattern, slowing of the rate, an increase in loudness, a shift in pitch level, and a disintegration of rhythm. In particular, the effect of delayed side-tone upon vocal rate, intensity, and rhythm has been investigated by Lee (*1–3*) and by Black (*4*). Lee states that the phenomenon is critical with regard to time of delay and volume of the feedback. He mentions that a one-quarter second delay is necessary for the effect to be prominent and that "the volume of the delayed feedback must be sufficiently high to dominate the sound of the subject's voice which he hears through bone conduction."

Black found that even the smallest delay produced a significantly reduced oral reading rate. He noted that rate decreased with increasing time of delay from 0 to .18 sec but that beyond this point, up to a .35-sec delay, the reading rate tended to increase—not returning to normal level, however.

It was the purpose of the present investigators to check Lee's assumption that the effect is critical with respect to the volume of the feedback and at the same time to investigate the possibilities of using the results in constructing a test for the detection of bilateral auditory malingering and/or psychogenic deafness.

In the present investigation a Presto RC-1024 tape recorder was used at a tape speed of 15 in./sec, which provided for a side-tone delay of approximately .14 sec. The output of the tape recorder was led through an appropriate attenuation network to PDR-8 headphones in a sound-isolated room. A volume unit meter enabled the operator to maintain a relatively constant input to the listener's ear. Thirty speech pathology students, with a reasonable degree of sophistication in speech and hearing problems, were chosen as subjects and were divided at random into three groups. Ten listeners received feedback at approximately 35 db above a speech threshold, 10 at 55 db, and 10 at 75 db. Audiometric evaluation of all of these students had revealed that they possessed hearing acuity for pure tones within a normal range.

Speech materials to be read consisted of a 100-word passage taken from *Robinson Crusoe* and modified for ease of reading. Subjects were allowed 5 min to acquaint themselves with the passage before entering the test situation.

The procedure employed was to ask each speaker-listener to read the passage three times. For the first reading the only instruction given was to read the passage in a normal manner. For this condition, although headphones were used and speech was recorded and timed by means of a stop watch, no feedback was employed.

For the second reading the subject was instructed to read the passage as nearly as possible like the first reading. At this time the problem of malingering was explained to him and, although he was not informed of the precise nature of the present test, he was told that he should attempt to keep from being influenced by anything he should hear, since he was to feign deafness. He was told that a deaf person would not respond to any sounds coming from the phones and would therefore read the passage the second time in the same way he had the first time. He was further told that he was to try to "beat the test" by not allowing any difference between the readings. Again this reading was recorded and timed, as well as being fed back at one of the predetermined intensity levels.

For the third reading the subject was given similar instructions but, as in the case of the first reading, no feedback was used. The third reading was employed as a measure of reliability or, in this case, constancy of reading rate. It was hypothesized that, in consequence of increasing familiarity with the material, each successive reading would take less time if the experimental condition had not been imposed; therefore any retardation of rate in the second or experimental reading would be a conservative estimate of the effects of the delayed side-tone as measured by reading time.

1. In order to determine the reliability of duration of repeated readings, t tests were applied to evaluate the significance of the differences between readings 1 and 3. For all three groups these were nonsignificant. The standard error of the mean differences ranged from .15 sec to .41 sec for the three groups, with an average reading time of approximately 27 sec.
2. As readings 1 and 3 are not significantly differ-