# Technical Papers

## The High Ascorbic Acid Content of the West Indian Cherry

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The West Indian cherry (*Malpighia punicifolia* L.), commonly called "acerola" in Spanish, is a small tree native to tropical and subtropical America. Its fruit is fleshy and drupaceous, bright red in color when ripe, and possessing an agreeably acid taste. Each of the cherries weighs about 5 grams; nearly 80 per cent of their weight is edible.

As far as we have been able to find out, the three richest natural sources of ascorbic acid known to date are the rose hips (*Rosa* sp.) (*4*), the "mirobalán" (*Phylanthus emblica* L.) (*3*), and the guava (*Psidium guajava* L.) (*5*). They contain about 1,700, 800, and 300 mg., respectively, of ascorbic acid per 100 grams of edible matter.

Utilizing the assay method of Bessey and King (*2*) (2,6-dichlorophenol indophenol titration), we found that the West Indian cherry contained within the high limits of 1,000–3,300 mg. of ascorbic acid per 100 grams of edible matter. The variation in its ascorbic acid content seems due principally to its degree of ripeness, the content being highest in the green fruit and lowest in the ripe.

TABLE 1

| Degree of ripeness and color of fruit | No. of samples* | pH of juice | Range mg. ascorbic acid per 100 gram of edible matter | Average mg. ascorbic acid per 100 gram of edible matter |
|---|---|---|---|---|
| Unripe—green ... | 4 | 3.35 | 2,516–3,309 | 2,963 |
| Medium ripe—yellow green with red spots ...... | 3 | 3.35 | 2,481–3.048 | 2,787 |
| Fully ripe—red .. | 4 | 3.35 | 1,030–2,700 | 1,707 |

* Each sample consisted of at least 15 cherries.

To confirm fully the presence of ascorbic acid in the West Indian cherry, the acid was isolated from the juice by a method not materially different from that utilized by Banga and Szent-Gyorgyi (*1*) in their study of peppers. Briefly, it is as follows: The ascorbic acid is precipitated from the juice in the form of the lead salt on the addition of neutral lead acetate, followed by enough ammonium hydroxide to bring the pH to 8.3–8.5. The lead salt thus obtained is decomposed with concentrated HCl, the supernatant acid mixture then concentrated *in vacuo,* and the ascorbic acid, present therein, extracted with acetone. The acetone extracts are mixed wih *n*-butyl alcohol, and the acetone is evaporated *in vacuo.* Ascorbic acid remains in the *n*-butyl alcohol and crystallizes out of it, after standing at a temperature of 0° C. for three days.

A gram and a half of pure crystals was obtained out of 275 ml. of the juice. These crystals had a melting point of 191–192° C.; a mix melting point with synthetic *l*-ascorbic acid, 189–191° C. Equal weights of the synthetic and natural product decolorized, to the same extent, equal volumes of indophenol reagent. The specific rotation in water of the synthetic and the natural substances was also similar. It is quite apparent that the substance obtained from these cherries is pure *l*-ascorbic acid.

It would seem that the West Indian cherry is one of the richest, if not *the* richest, edible fruit source of ascorbic acid so far described in the literature.

### References

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## Motherless Males From Irradiated Eggs[1]

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"Androgenesis" (*6*) was first demonstrated by Hertwig (*3*), who found that, when amphibian eggs are heavily irradiated and then fertilized by untreated sperm, abnormality in development is *inversely* proportional to intensity of treatment. Embryos from such eggs have nuclei smaller than normal, and these nuclei were thought to be haploid and descendants of the sperm nucleus. Packard (*4*) irradiated Chaetopterus eggs in first meiotic metaphase and obtained clear cytological proof that the untreated sperm nucleus in such eggs can form the first cleavage figure and its successors. Dalcq (*1*) checked Hertwig's results and added cytological proof of androgenesis in Amphibia.

It is uncertain whether androgenetic larvae in any of these experiments would develop into adults. As

[1] Aided by a grant from the Rockefeller Foundation.

Fankhauser (*2*) has pointed out, "In the thousands of experiments which have been performed up to the present time, not a single haploid animal has been raised to a stage approaching sexual maturity." He is referring, of course, to animals in which all individuals are normally diploid. Normal, mature, androgenetic haploids might be expected to occur in species with haploid males, such as bees or wasps. During many years of intensive breeding of the parasitic wasp *Habrobracon juglandis,* several hundred mosaic individuals have been found among which 11 only (*5*) have had regions which were unquestionably paternal in origin. Among the progeny from crosses of dominant females by recessive males (condition in which androgenetic males can be identified) only five males have been reported showing genetic traits exclusively paternal with no traces of mosaicism. None of these was given a breeding test. A sixth, with "glass" eyes, bred as "glass," showing the gonads to be derived from the sperm nucleus. Thirteen of these 17 individuals were produced by females of one stock, and none was found in crosses from the stocks used in this experiment. Spontaneous androgenesis is, therefore, very rare, even in a species with normal male haploidy.

In a recent experiment, unmated females of an inbred wild type stock of Habrobracon were heavily X-rayed,[2] then mated to untreated males with recessive mutant traits. The surviving progeny (from eggs treated in first meiotic prophase) included among the expected wild type males (haploid and maternal) and females (diploid and biparental) a few males normal in structure and behavior and with the recessive mutant traits (haploid and paternal). Breeding tests demonstrated that their gonads were also paternal in origin, and the use of body color differences helped to show that they were not mosaic. Their normal fertility was proof of their haploidy, since diploid males in this species are almost completely sterile. Table 1 summarizes the results.

The cytological phenomena underlying the production of these motherless males remain to be investigated. Two possibilities suggest themselves: (1) The irradiated maternal chromosomes may be retarded in movement and fail to reach the male pronucleus in time for first cleavage or, having reached it, are soon eliminated so that the haploid paternal chromosome complement forms the embryo. The observations of Packard and of Dalcq demonstrate this type of behavior. (2) The fusion nucleus may fail to function, hampered by its irradiated chromatin, and, when dispermy occurs, the free sperm may form the embryo.

In egg X-rayed with lethal dose (*ca.* 45,000 r) and allowed to develop parthenogenetically, about 1.1 per cent die at first cleavage, while the remainder develop well beyond this stage, often to blastoderm. In normal eggs about 1 per cent show dispermy. Either of these facts would seem to limit the production of androgenetic males to about 1 per cent, but survival percentages may not be dependable criteria of modes of origin because of differential viability.

[2] By L. R. Hyde at the Marine Biological Laboratory, Woods Hole, Massachusetts.

TABLE 1

| Dose in r units | Number females treated | Progeny: Wild type males | Wild type females | Recessive males | Per cent andro-genesis |
|---|---|---|---|---|---|
| 28,000 .... | 378 | 82 | 341 | 15 | 4.21 |
| 29,300 .... | 43 | 8 | 10 | 3 | 23.07 |
| 42,000 .... | 202 | 1 | 7 | 1 | 12.50 |
| Controls .. | 17 | 160 | 467 | 0 | 0.0 |

The production of these normal, sexually mature individuals from the sperm nucleus is of theoretical interest from two aspects. It adds evidence to a concept which now seems to need little—that hereditary traits are carried by the nucleus—and appears to strengthen the point of view that X-ray injury, at least up to lethal dose, is directly chromosomal, since untreated chromosomes can function normally in the heavily treated cytoplasm of an egg whose own chromosomes are so seriously injured as to be unable to function.

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## Diabetes Produced by Feeding Alloxan to Cats[1]


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It is well established that diabetes may be experimentally induced in animals by *injection* of an appropriate amount of alloxan solution intravenously, intraperitoneally, intramuscularly, or subcutaneously. In studying the introduction of alloxan into the alimentary canal without subjecting the animal to the effect of an anesthetic or an operative procedure, we have discovered that diabetes can be produced by *feeding* alloxan to cats. Furthermore, in addition to the destructive effect on the beta cells of the pancreas, we have noticed injury to the adrenal cortex, the anterior lobe of the pituitary, the liver, and the kidneys.

*Method.* One part of alloxan was freshly mixed

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