

a phosphor activated by radioactive emanations or by some luminous dial paint.

These shields have been used when studying the biological effects of radiogallium (Ga^{72}) which has an unusually energetic spectrum (β 3.1 mev, γ 2.5 mev). Persons wearing film badges and pocket electroscopes on the body and on the hands have received less than 20 milliroentgens (mr) total body radiation and less than 40 mr on the hands, when injecting solutions of 0.4 mc/ml activity, in quantities up to 4 mc per injection and a total of 25 mc.

The difficulties encountered in using shields of the types described are those of manipulation, due to their size and weight, particularly of the metal shields.

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Erroneous Ascorbic Acid Values Resulting from Interference by Anthocyanins

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The determination of ascorbic acid in anthocyanin-containing plant products presents special problems. If the usual indophenol dye reduction methods are used, the color due to anthocyanin is very similar to that of the dye in acid extracts. In highly colored extracts, it then becomes desirable to eliminate this color. This is easily accomplished by a xylene extraction of the excess dye following the reaction of the dye with ascorbic acid (1, 2, 5). Anthocyanins are not extracted by xylene under these conditions. This procedure has been used in the analysis of colored plant materials (3, 5).

We attempted to use a xylene extraction method (2) for determining the ascorbic acid content of red beets. As previous workers had reported, it was found that the anthocyanin, betanin, was not extracted by xylene and hence did not interfere with the determination due to its color *per se*. However, analytical difficulties of another sort were encountered. It was found, for example, that aliquots of a single metaphosphoric acid extract of beet hypocotyl did not check if the aliquots were of different sizes. Also, similar metaphosphoric acid extracts containing different amounts of tissue per unit volume failed to give comparable results. When aliquots of such extracts were treated with formaldehyde (6), the capacity to reduce the dye was decreased to only about one-half

its original value. Ascorbic acid is rendered nonreactive under these conditions, and in the absence of interfering substances formaldehyde-treated aliquots no longer reduce the indophenol dye. Hence, it was concluded that these extracts contained appreciable quantities of reducing substance (or substances) other than ascorbic acid. It was observed that this residual reducing capacity was closely correlated with the color of the metaphosphoric acid extracts (coefficient of correlation = +0.724, $n=81$).

These observations led to an investigation of the extent of similar interference in other anthocyanin-containing products. In fresh fruits the amount of interference was found to be small; but in a number of fruits stored for months at 0° F, during which time the ascorbic acid largely disappeared, the amount of interference became relatively important.

In order to determine whether or not anthocyanins were the cause of the interference, an attempt was made to isolate various anthocyanins in more or less pure form and to study their reaction with the indophenol dye. It was found that betanin could be obtained in a somewhat concentrated form as follows: Fresh beets were sliced and frozen and the juice was pressed out; this juice was frozen and dried *in vacuo*. When the resulting powder was suspended in water and adjusted to about pH 3.5, the anthocyanin was precipitated by adding ethyl alcohol to give a concentration of about 80%. The precipitate was washed with 95% ethanol and then with acetone by centrifuging, and dried *in vacuo*. The resulting red powder was readily soluble in water and it reacted with the indophenol dye in much the same way as the interfering material present in extracts from fresh beets. Its reaction was not prevented by formaldehyde. Betanin isolated according to the procedure of Pucher *et al.* (4) reacted in a similar manner. Thus, it appears evident that the interfering material in extracts from fresh beets is the anthocyanin, betanin.

In view of these results, it seemed desirable to determine the antiscorbutic activity of the concentrated anthocyanin by means of a bioassay. Betanin was prepared from lyophilized beet juice by alcoholic precipitation as outlined above. This material was then assayed chemically and was fed to guinea pigs at the rate of 4 mg daily of apparent ascorbic acid (chemical assay) per animal. The bioassay selected was the depletion technique as used by Tressler, Mack, and King (7). From the results obtained it was concluded that the anthocyanin concentrate had no antiscorbutic activity for the guinea pig.

These results will be reported in greater detail elsewhere.

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