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Interference by sucrose in the estimation of ribonucleic acid by the orcinol method

A standard procedure for the identification of ribonucleic acid (RNA) in tissue extracts is the orcinol method[1,2] which has been adapted by numerous workers for the quantitative estimation of RNA (see DISCHE[3]).

It has been pointed out on several occasions that sugars other than ribose interfere in the reaction. The orcinol method is, in fact, quite unspecific for ribose, producing a green colour with other pentoses, and varying shades of green-brown with hexoses[4] and disaccharides[5]. The absorption spectra of several sugars in the orcinol reaction have recently been published[6].

Of the many media tried and being used in biochemical studies on sub-cellular fractions, aqueous sucrose (usually 0.25 *M*) is by far the commonest; very often RNA estimations are performed on tissue extracts prepared from sucrose suspensions. As traces of 0.25 *M* sucrose produce a heavy brown precipitate under the conditions of the orcinol method, it is obviously of prime importance that sucrose should be entirely absent from the final extract containing RNA.

With the SCHNEIDER[7] procedure for extracting RNA, this means, in effect, that the nucleic acids and proteins precipitated by cold trichloracetic acid (TCA) must be washed free of sucrose before the nucleic acids are extracted by hot TCA at 90°. However, it is possible, particularly if the alcohol-ether extractions are omitted, for sucrose to be left behind with the nucleoprotein precipitate and to be subsequently taken up into the nucleic acid extract. These traces of sucrose seriously affect the extinction reading at 670 mμ and the investigator would probably be unaware of it except in cases of extreme contamination. This communication describes a routine method for checking sucrose contamination, and also how to apply a correction if it occurs.

In all determinations, readings were obtained using a Unicam SP-500 spectrophotometer, and cells of 1 cm path length. The volumes of solutions used were as follows: 3 ml 1% $FeCl_3$ in conc. HCl; (2 + x) ml water; 0.3 ml orcinol (10% in absolute ethanol); (1 — x) ml of the test carbohydrate solution. The mixtures were heated for 45 min in a boiling water-bath. 1 ml absolute ethanol was added after boiling to tubes containing sucrose. Under these conditions, sucrose gives maximum absorption at 430 and 530 mμ; there is also a small peak at 660 mμ. RNA solutions, on the other hand, follow the absorption spectra of pentoses very closely and give maxima at 420 and 670 mμ with a minimum at 520 mμ[6].

It was found that for both sucrose and RNA solutions the extinctions at 520 and 670 mμ follow Beer's Law. This means that the ratio of E_{670}/E_{520} is constant for both sugars over the wide range of concentrations studied. For sucrose this ratio was found to be 0.321 whereas for RNA solutions it was 4.10. Thus, in the determination of RNA by the orcinol procedure, routine measurement of the ratio of E_{670}/E_{520} eliminates the uncertainty as to whether sucrose contamination is occurring. A similar procedure has been applied by BROWN[4] for eliminating hexose interference in pentose estimations.

Using mixtures of RNA and sucrose of known concentration, it was found that strict additivity of the extinctions either at 520 mμ or at 670 mμ could not be obtained. A slight deviation from additivity in mixtures appears to be the general rule (*cf.* [8]). At 520 mμ the deviation

from additivity was + 8% and at 670 mμ + 13%. In other words, sucrose–RNA mixtures give readings at 670 mμ on the average 13% higher than would be expected from the individual extinction values. However, to the first approximation, one can neglect these deviations when applying a correction for the interference of sucrose in the orcinol reaction.

Regression lines were calculated for the dependence of the extinction values at 520 mμ and 670 mμ on both sucrose and RNA concentrations. Thus, equations of the following form were obtained:

$$E^{\lambda} = K_x^{\lambda} \cdot C_x$$

where E^{λ} = extinction at wave-length λ mμ for a 1 cm path-length;
K_x^{λ} = constant for component x at wave-length λ mμ;
C_x = concentration of component x in μg added to a final volume of 6.3 ml.

The values of K_x^{λ} found at the two wave-lengths for sucrose and for RNA were:

	670 mμ	*520 mμ*
Sucrose	$0.586 \cdot 10^{-3}$	$1.824 \cdot 10^{-3}$
RNA	$4.150 \cdot 10^{-3}$	$1.012 \cdot 10^{-3}$

Therefore, assuming a strict additivity of extinctions at both 520 mμ and at 670 mμ the following equation can be obtained correcting the extinction at 670 mμ for the presence of sucrose in RNA estimations:

$$\text{Concentration of RNA} = \frac{3.11.\ E_{670} - E_{520}}{11.92} \times 10^3\ \mu\text{g in final volume of 6.3 ml.}$$

The efficiency of this correction is illustrated by the Table I where the extinctions at 520 mμ and 670 mμ of sucrose–RNA mixtures of known concentrations are shown. The sucrose correction reduces the error in the determinations from between 30–70% to under 5%.

In common with the work of BROWN[4] on hexoses, it is suggested that routine measurement of RNA colours at 520 mμ as well as at 670 mμ will remove the uncertainty of interference by sucrose and, providing that the interference is not gross, will also correct for it.

TABLE I

INFLUENCE OF ADDED SUCROSE ON THE DETERMINATION OF RNA BY THE ORCINOL METHOD

RNA added (μg)	*Sucrose added (μg)*	E_{670}	E_{520}	*RNA found (uncorr.)*		*RNA found (corrected)*	
				(μg)	*error (%)*	*μg*	*error (%)*
65.5	97.6	0.342	0.272	83.0	28	66.5	1.5
65.5	195.2	0.400	0.493	96.0	48	63.1	3.6
65.5	292.8	0.453	0.681	108.0	65	62.0	5.3
131.0	390.4	0.818	0.963	196.0	50	132.6	1.2
131.0	488.0	0.865	1.140	206.0	57	130.0	0.7
131.0	585.6	0.928	1.330	220.0	68	131.0	0.0

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