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A Modified Orcinol Reaction for RNA Determination (*)

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The Schneider orcinol technic for pentose estimation was modified by replacing ferric chloride with cupric acetate in glacial acetic acid as catalyst, to make the test more sensitive and less DNA-reactive. In addition, data were obtained showing that human plasma contains no detectable nucleic acid by the spectrophotometric determinations applied.

AMONG the various colorimetric methods for RNA estimation, the orcinol reaction of Mejbaum as modified by Schneider (1) remains the most sensitive and commonly employed technic for purine-bound ribose quantitation. Later attempts that were made to estimate pyrimidine nucleotides and nucleosides (2, 3) have also been based on the color reaction of ribose with orcinol and ferric chloride, which still has the disadvantage of being nonspecific for ribose estimation.

The present paper describes a modification which is more sensitive, more rapid, and less affected by DNA, which usually is present in biologic preparations extracted by either trichloracetic acid (TCA) or perchloric acid (PCA).

Methods

To 0.6 ml of the acid-hydrolized tissue extract (4), with a RNA content of 50-200 μg/ml, are added 0.4 ml of a stock solution containing 0.5% (w/v) cupric acetate in glacial acetic acid, 2 ml distilled water, and, finally, 3 ml of the reagent prepared by dissolving 1 g orcinol in 100 ml concentrated hydrochloric acid. Into two tubes marked *Blank* and *RNA standard* are added, respectively, 0.6 ml of a 0.5 N PCA solution, and 0.6 ml of the working standard solution containing 10 mg reagent grade RNA (Nutritional Biochemicals Corporation) in 100 ml of 0.5 N PCA. Into both tubes are then added 0.4 ml of the cupric acetate solution, 2 ml distilled water, and 3 ml of the reagent, as added to the *Test* tube.

The content of the tubes are mixed thoroughly, and the tubes are kept in a vigorously boiling water bath for a period of 10-15 min. The bluish-green color which develops within 5 min. of boiling is read at 660-670 mμ in a Coleman Jr. spectrophotometer, using 19 × 150 mm. cuvets.

The above-mentioned method and the Schneider procedure were applied to guinea pig liver extract (4), freshly drawn heparinized human plasma of normal

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and pregnant subjects, and to sperm DNA purchased from Nutritional Biochemicals.

Results and Discussion

After making corrections for DNA contribution, RNA value of the liver extract was found to be identical when using either method.

Cupric acetate reagent produced no bluish-green color with the PCA- precipitable fraction of human plasma, although upon prolonged heating, the reaction mixture turned to an unspecific brownish color due to the presence of unknown interfering substances. Also, DNA could not be detected in the plasma extract using the diphenylamine reaction of Burton (5). These findings provide data on the absence in human plasma of nucleic acids, at least to a detectable level, regardless of the conflicting reports so far presented (6, 7). Table 1 shows the absorbance values obtained for 60 μg RNA and 200μg DNA determined by both the Schneider and the present methods.

The color in cupric acetate tube develops within 5 min. of boiling, while the green color does not become visible with 60μg of RNA used, even after 20 min. when ferric chloride is used in the reagent. It turns to a light green under our experimental conditions, but only after cooling the tubes in tap water.

According to the statistical comparison (8, 9) developed in Table 1, there exists a significant difference between the mean ratio of the two methods: Certain quantities of RNA and DNA give higher and lower absorbance readings, respectively, in the present technic than in that of Schneider. It could also be concluded that substitution of cupric acetate/glacial acetic acid for ferric chloride renders the test 3.5-5.5 times more sensitive, the sensitivity being calculated by dividing $\frac{A_{RNA}}{A_{DNA}}$ of cupric acetate (mean ± 1 S.D.) by $\frac{A_{RNA}}{A_{DNA}}$ of the $FeCl_3$ (mean $\pm$ 1 S.D.) method.

Finally, the cupric acetate modification consumes far less salt, and calls for a lower correction factor for the contribution of DNA to the color obtained in the orcinol-RNA reaction. However, the presented method suffers from the interference of such substances as glucose, deoxypentose, etc, as do the other technics that are based on the orcinol reaction.

Table 1. RNA and DNA Absorbance Readings by the Ferric Chloride and Cupric Acetate (CuAc) Technics

	A/60 µg RNA		*A/200 µg DNA*		*RNA : DNA ratio*		
Experiment No.	*$FeCl_3$*	*CuAc*	*$FeCl_3$*	*CuAc*	*$FeCl_3$*	*CuAc*	*Difference between ratios*
1	0.1990	0.3363	0.0482	0.0177	4.12	19.00	+14.88
2	0.2110	0.3440	0.0444	0.0155	4.75	22.19	+17.44
3	0.1855	0.3400	0.0440	0.0167	4.21	20.26	+16.15
4	0.1973	0.3000	0.0458	0.0177	4.30	16.95	+12.65
5	0.1838	0.3210	0.0555	0.0167	3.31	19.22	+15.91
6	0.1860	0.3138	0.0456	0.0200	4.07	15.69	+11.62
7	0.2007	0.3520	0.0580	0.0212	3.46	16.60	+13.14
8	0.1956	0.3520	0.0412	0.0223	4.74	15.78	+11.04
9	0.1922	0.3280	0.0434	0.0166	4.42	19.75	+15.33
10	0.1990	0.3490	0.0444	0.0188	4.48	18.56	+14.08
Mean	0.1950	0.3336	0.0470	0.0183	4.18	18.41	+14.22
S.D.*	N.D.†	N.D.	N.D.	N.D.	0.47	2.12	2.08

The t test: 6.8. The value significant at 0.1% level. Method of t (paired observations) calculation: $t = \frac{\bar{d}}{s\bar{d}}$.

* Method of standard deviation (S.D.) calculation (9): $s = \sqrt{\frac{\Sigma y^2 - (\Sigma y)^2/n}{n - 1}}$

† N.D., not determined.

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