IMPROVEMENT ON THE PRESCOTT-JONES METHOD FOR THE COLORIMETRIC ANALYSIS OF UREIDO COMPOUNDS

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Improvements on the Prescott-Jones Method for the Colorimetric Analysis of Ureido Compounds

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ABSTRACT

The colorimetric assay procedure of Prescott and Jones [(1969) Anal. Biochem. 32, 408-419] has been modified to make the standard curve more consistently linear. Improved results are obtained when the assay tubes are exposed to yellow light at an intensity of 45-50 footcandles (480-540 lux) during color development. Somewhat longer incubation times are required for color development with yellow light.

Several years ago Prescott and Jones (1) described a sensitive and convenient procedure for the colorimetric analysis of ureido compounds such as carbamyl aspartate, citrulline, and urea. More recently, Savage et al. (2) eliminated 2-mercaptoethanol interference with the assay by treating reaction mixtures with N-ethylmaleimide before the addition of the color reagent, thus making the method more suitable for enzyme studies. Under some circumstances, however, we have noted that standard curves of absorbance vs. the concentration of ureido compounds are nonlinear. We have found that the quality and quantity of light used during color development can affect the linearity of the standard curve. We describe simple modifications of the assay procedure which correct this difficulty and which further increase the potential utility of the assay.

METHODS

The basic assay conditions and procedures have not been altered radically. The original reagent concentrations have been reduced slightly in order to save chemicals:

(1) Antipyrine /H_2SO_4 reagent: 4 g/ℓ antipyrine (1,5-dimethyl-2-phenyl-3-pyrazolone) in 40% (v/v) sulfuric acid.

(2) Oxime reagent: 0.5 g diacetylmonoxime in 100 ml of 5% (v/v) acetic acid.

The color reagent employed in the assay still consists of two parts antipyrine/H_2SO_4 reagent and one part oxime reagent.

The most important modification involves the nature of the light source used during color development. Although regular fluorescent illumination may sometimes be employed successfully, best results will usually be obtained if yellow light of relatively low intensity is used instead. The light source consists of two 15 to 40 watt cool white fluorescent bulbs. The lamps are

covered with a single layer of yellow cellophane so that the assay tubes are exposed only to yellow light. The lamps must be positioned above the 60°C water bath so that the assay tubes are exposed to light of an intensity of 45-50 footcandles (480-540 lux).

The utilization of a modified light source necessitates slight alterations in the incubation time required for color development. The following incubation times have been used with the method I procedure described by Prescott and Jones (1969): 90 min. for citrulline, 120 min. for carbamyl aspartate, and 180 min. for urea.

RESULTS AND DISCUSSION

Using the above modifications of method I, linear standard curves are obtained with only slightly less sensitivity. The 1.0 cm absorbance values at 466 nm per 0.10 μmole in the standard 1.0 ml sample volume as read on a Bausch and Lomb Spectronic 70 are 0.31 units (carbamyl aspartate), 1.06 units (citrulline), and 0.94 units (urea). Color development with yellow light has also been employed successfully in method II (dark incubation before exposure to yellow light).

With the incorporation of these changes the colorimetric assay is quite sensitive, reproducible, and simple to carry out. We have employed both 15 watt bulbs (General Electric F15T8-CW) and 40 watt bulbs (Phillips F40T/CW) with equivalent results. The source of the yellow cellophane also does not seem to alter the results significantly, although all types we tried did effectively block wavelengths shorter than 450 nm. It is important to both expose the tubes to the light for an adequate length of time and not use yellow light of too great an intensity. Standard curves will become nonlinear if the light intensity reaches 65 footcandles (700 lux).

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REFERENCES

1. Prescott, L. M. and Jones, M. E. (1969) Anal. Biochem. 32, 408-419.
2. Savage, C. R., Schumer, J. M., and Weinfeld, H. (1973) Anal. Biochem. 53, 431-440.

FOOTNOTES

1. To whom requests for reprints should be addressed.