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Research Article

The Estimation of Vitamin A Aldehyde in Egg Yolk by a New Method

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ABSTRACT

A new simple colorimetric procedure is discovered for the estimation of retinal in egg yolk. This method does not require chromatographic separation of the other retinoids or carotenoids. After extraction by alcohol and petroleum ether, the petroleum ether part is evaporated to dryness by passing carbon-di-oxide; to it thiobarbituric acid reagent was added and mixed well. Immediately thiourea reagent was mixed and reading was taken after 30 minutes by a colorimeter at 530 nm. Calculation of retinal was done from the standard curve prepared earlier.

INTRODUCTION

In the ripe eggs of all species of marine teleost fish so far examined, retinal(Vit A *aldehyde*) is the predominant form of vitamin A, and in some species it represents 90% of the total vitamin Aactive material in the eggs . Hen's eggs also contain retinal(Plack P A,1963; Plack P A et al., 1961; Plack P A et al., 1959). The predominance of retinal in fish eggs suggests that it may be of importance in the development of the embryo.The role of vitamin A aldehyde (retinene) as the chromophore of visual pigments has been established through the efforts of several investigators (Wald G.,1935; Pitt G., 1985; Wald G et al., 1956). Commonly used methods for the estimation of vitamin A aldehyde were originally devised for estimation of vitamin A. In the Carr-price test (Krinsky N I, 1958; Dowling J E, 1960) a transitory blue colour is produced in a chloroform solution of vitaminA aldehyde and antimony trichloride. In the reaction with 1,3-dichloro-2-propanol,a green colour is obtained with vitamin A aldehyde(SOBEL A E et.al., 1947; Polland C J et al., 1959).When these tests are used, corrections may be required for accompanying vitamin A. Ultraviolet absorption measurements have also been used for estimation of vitamin A *aldehyde* (Krinsky N I, 1958; Dowling J E, 1960; SOBEL A E et al., 1947; Polland C J et al., 1959; Ball S et al., 1949; Ball S., and Morton R A., 1949) . However, the analysis complicated by differences in the spectral properties of the various *cis* and *trans* isomers when more than one isomer is present. Colored products are obtained by reaction of vitamin A aldehyde with amines (Polland C J et al., 1959; Ball S et al., 1949) concentrated mineral acids (Ball S., and Morton R A., 1949) but the suitability of these reagents for the quantitative determination of vitamin A aldehyde has not been demonstrated. In this present investigation, a new colorimetric method is described for the estimation of vitamin A *aldehyde* in egg yolk.

MATERIALS & METHODS

Materials

All-*trans* vitamin A *Aldehyde,* Thiourea and Thiobarbituric acid were obtained from sigma chemical company.

Reagents

Absolute Alcohol, Thiourea Reagent: Dissolve 4g of Thiourea 100ml of glacial acetic acid and filter the solution through glass wool. Thiobarbituric acid Reagent: Dissolve 600mg of Thiobarbituric acid in 100ml of absolute ethanol. Filter the solution and store it in a refrigerator. Vitamin A *Aldehyde* Stock solution: Dissolve 10mg of all-*trans* Vitamin A *Aldehyde* in 100ml of absolute Ethanol. Shield the solution from light with aluminium foil and store it in a refrigerator.Vitamin A *Aldehyde* Standard solution: Mix 1ml of stock solution is dilute to 25ml of Ethanol. The solution is prepared each day. All solutions may be stored for 40 days.

Estimation of Vitamin A Aldehyde

One millilitre of egg yolk was taken in a centrifuge tube. To it 5ml of absolute alcohol and 5ml of petroleum ether (BR 40oC – 60oC) were added and mixed well. Then it was allowed to settle or if required centrifuged for 5 minutes at 1000 RPM. The petroleum ether layer was taken in a test tube and it was evaporated to dryness by passing CO2 through the petroleum ether. In this dried test tube 3ml of absolute alcohol, 1ml of Thiourea reagent and 1ml of Thiobarbituric acid Reagent were added and mixed by the Vortex mixer. The solution was kept in the dark for 30 minutes and colour is measured by a colorimeter at 530 nm filter.

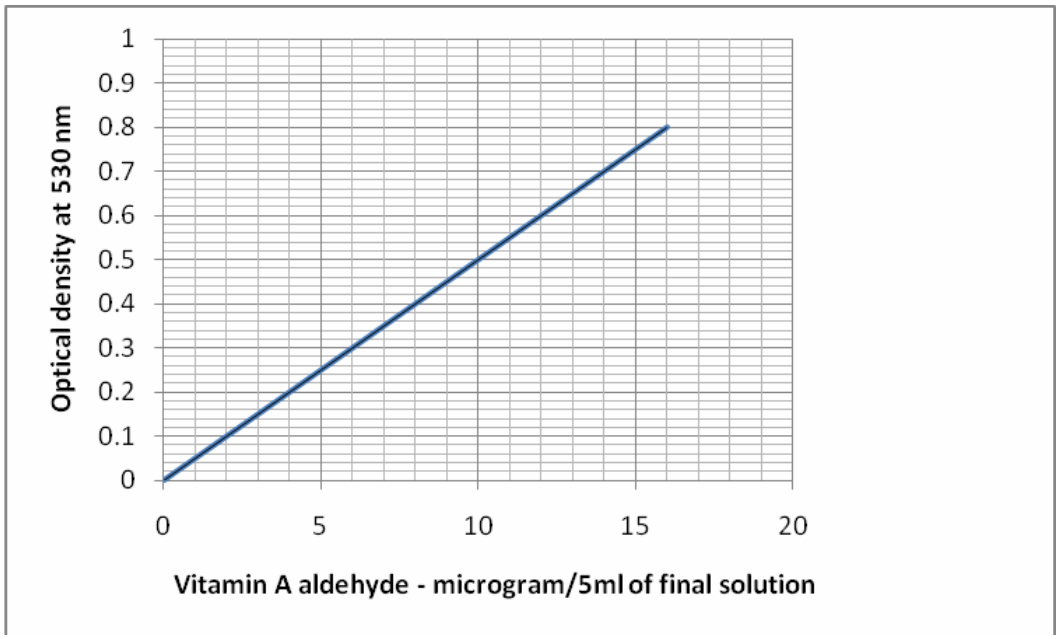

Figure 1: Standard curve for Vitamin A aldehyde:

RESULT & DISCUSSION

A standard carve was prepared with the same method and it is given separately. At 530 nm two micrograms with the reaction mixture of total 5 ml reads 0.1 optical densities and the curve was linear.The mean retinal found in the egg yolk (hen) was found to be 3.36 µg/G of yolk.Contamination of retinol, retinyl esters or carotene cannot affect the result. The method can be applied to any tissue containing retinal or can be used for estimation in the drugs.The reaction mixture was orange coloured after 30 minutes in the dark..

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