BRIEF COMMUNICATIONS

USE OF THE LUMINESCENCE METHOD FOR DETERMINATION OF ADRENALINE AND NORADRENALINE IN LIVING ORGANISMS

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The luminescence method for quantitative determination of compounds has been successfully employed in biochemical investigations [1]. This technique is most accurate for determination of such catecholamines as adrenaline and noradrenaline (A and NA). In determination of small amounts of catecholamines by the fluorescence method, high requirements are imposed on the purity of the reagents, the elimination of diffused light, etc. In this connection, it should be noted that the results of determination of tissue catecholamine content are ambiguous.

The present article describes a modified method for measurement of the amounts of A and NA in animal tissue that makes it possible to eliminate the influence of a number of factors on determination accuracy. We employed A and NA preparations produced by Osinskaya's method [2]. The luminescence measurements were made in an apparatus based on an ISP-51 spectrograph [3]. The recording unit consisted of an FÉU-38 photomultiplier, a narrow-band amplifier, and a synchronous detector. The excitation source was an SVD-120A bulb with an UFS-3 filter.

The method used to prepare the reagents did not preclude the possibility that the test solution contained noticeable amounts of luminescent impurities. Use of the usual methods for recording the integral luminescence light to determine the catecholamine content therefore did not make it possible to take into account the contribution made by the impurities, which affected the measurement results. In order to evaluate and monitor the luminescent impurities, we measured the luminescence spectra of the test preparations and the control solution. It can be seen from Fig. 1 that the latter makes a large contribution to the luminescence spectrum of A, so that such analysis of the spectra is fully justified.

Moreover, the concentration of the test compounds varied within a range of three or four orders of magnitude with the proposed method; it was therefore necessary to vary the amplification constant of the recording circuit over the same range and to plot a standard curve for each amplification constant [4]. Use of the latter required a highly stable amplification factor. In order to eliminate this rigid requirement on the amplification constant, we introduced a standard light source: an MN-14 incandescent bulb supplied by a stabilized voltage source and subjected to previous break-in. The intensity of the light from the bulb was readily varied over a wide range and was unambigously related to the current passed through the bulb.

The area S under the histogram recorded for the luminescence of A or NA is related to the A or NA concentration C by the expression

$$S = MKC\int_{\lambda_1}^{\lambda_2}\varepsilon(\lambda)\,f(\lambda)\,d\lambda = \alpha_1 KC,$$

where M and K are the amplification factors of the FÉU and amplifier, $\varepsilon(\lambda)$ is the spectral sensitivity of the photocathode, and $f(\lambda)$ is the spectral density of the luminescence.

The signal amplitude U produced by the incandescent bulb over the spectral range λ_0 is

$$U = \gamma\Delta\lambda_0\varepsilon(\lambda_0)\,\eta(\lambda_0,\ I)\,MK = \alpha_2 K\eta(\lambda_0,\ I) = \alpha_2 K\eta(I)$$

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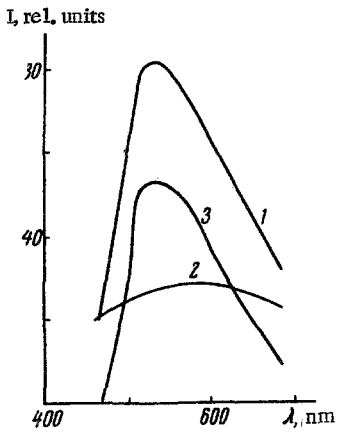

Fig. 1

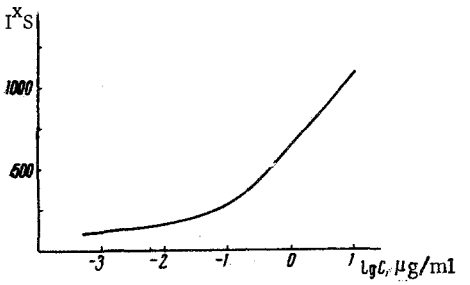

Fig. 2

Fig. 1. Luminescence spectra of adrenaline solutions prepared by Osinskaya's method. 1) Luminescence spectrum of third sample; 2) luminescence spectrum of second sample; 3) difference between spectra 1 and 2 (luminescence spectrum of adrenaline). The adrenaline concentration was 0.01 μg/ml.

Fig. 2. Standard curve for pulsed adrenaline hydrochloride solution between 0.0001 and 10 μg/ml.

with constant $\Delta\lambda_0$ and λ_0. Here $\eta(\lambda_0, I)$ is the spectral density of the bulb radiation at λ_0 and I; I is the current passing through the bulb. If U is constant, then $K = \alpha_2'\eta^{-1}(I)$ and

$$S = \alpha_1 C\alpha_2'\eta^{-1}(I) \quad \text{or} \quad S\eta(I) = \beta C.$$

The function $\eta(I) \approx I^X$, where x is determined experimentally. Hence it follows that the product $S \cdot \eta(I) = S \cdot I^X$ serves as a measure of C for a standard source. Figure 2 gives the standard curve obtained for adrenaline.

The procedure for determination of the unknown quantity C runs as follows: the value of K is varied to obtain the requisite amplitude of the measured luminescence spectrum; with this K, I is varied to achieve the equality $U = \alpha_2 K\eta(I)$. The product $S \cdot I^X$ is calculated and the value of C is determined from the standard curve.

The luminescence-band maxima obtained for A and NA in the present investigation (540 and 525 nm) are in good agreement with the data in the literature [5, 6].

In order to check the proposed method, we measured the concentrations of A and NA in a fluorometer and by our technique. The determinations were made in a trichloroacetic acid extract of tissue from the canine posterior mesenteric sympathetic ganglion. The results obtained were in good agreement ($\leq 3\%$).

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