APPARATUS FOR THE FLUOROMETRIC DETERMINATION OF SEROTONIN AND OTHER BIOGENIC AMINES

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The investigation of the mode of action and the metabolism of serotonin in normal and pathological processes is attracting the interest of a wide circle of research workers in the field of theoretical and practical medicine. The assay of this amine, however, entails some methodological difficulties. A considerable amount of the data relating to the serotonin content of organs and tissues has been obtained by the biological method, which is based on the ability of serotonin to stimulate contraction of the smooth musculature. The test objects used for the action of serotonin are the small intenstine or colon of the rat or guinea pig [1, 2], an isolated strip of rat stomach tissue [3], the isolated uterus of an estrogen-treated rat [4], and so on.

Biological methods, however, though relatively sensitive, do not always indicate definitely that the observed effect is due to serotonin, and not to associated agents. In addition, they are laborious and the accuracy of amine assay is low.

Much better in this respect are the physicochemical methods, particularly Bogdansky's spectrofluorometric method [5], which is based on the ability of serotonin to fluoresce in an acid medium when it is irradiated with ultraviolet light. Several modifications of this method have been suggested. Its extensive use is hampered by the absence of standard apparatus for measurement of fluorescence excited in the far ultraviolet (295 nm). In this paper we described an instrument based on a mass-produced Russian apparatus and with a minimum number of nonstandard components.

Figure 1 clearly shows that the absorption maxima of serotonin lie in the region of 280 and 295 nm, while the fluorescence maximum is at 550 nm. These are the wavelengths at which the fluorescence of the solution is excited and measured. Monochromatic excitation in the far ultraviolet and measurement of the fluorescence at a particular wavelength require the use of two monochromators. This not only makes the apparatus more expensive, but leads to a considerable loss of light, which is extremely undesirable in measurement of the weak fluorescence of dilute serotonin solutions. Hence, we decided to dispense with one of the monochromators and replace it with light filters. For several reasons, we considered it better to retain monochromatic excitation of the fluorescence and to measure the fluorescence of serotonin through a combination of filters. It is rather difficult to select a filter combination which satisfactorily isolates the 295-nm spectral region. It is quite impossible with glass filters, and liquid filters usually transmit a fairly broad spectral region (for instance, the well-known combination of $NiSO_4$ and $CoSO_4$ solutions transmit the 240- to 360-nm region [6]). A combination of a liquid of gas filter can be used to isolate the narrower region of 240–280 nm [7], which is suitable for excitation of the fluorescence of proteins and nucleic acids. It is not quite suitable for the excitation of serotonin, however, (see Fig. 1a), since it does not correspond to the absorption maximum, and this shorter-wave radiation may excite the fluorescence of impurities. At present, as far as we know, there are no mass-produced interference filters for isolation of the 295-nm region.

Thus, satisfactory isolation of the 295-nm exciting radiation requires the use of a monochromator. (We used a monochromator from the SF-4 spectrophotometer; see Fig. 2.) On the other hand, isolation of the spectral region near 550 nm does not present particular difficulties and can be effected with a filter com-

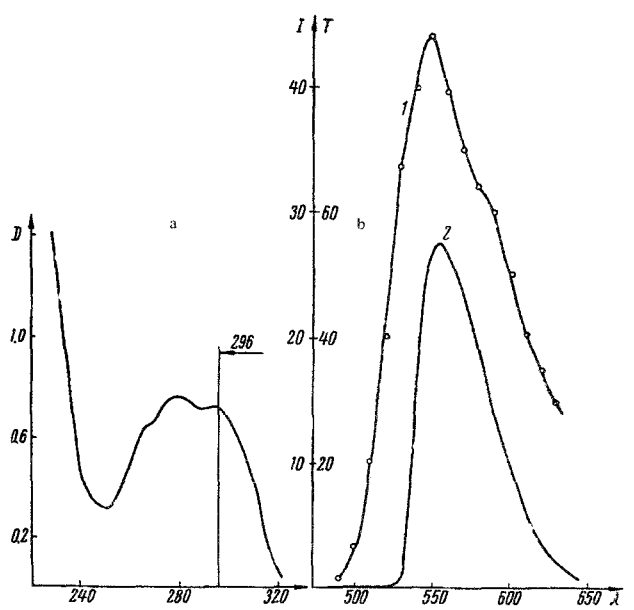

Fig. 1. Absorption (a) and fluorescence (b, 1) spectra of serotonin in 3 M HCl; 2) transmission of OS-11 + + SZS-21 filters. I is the intensity of the fluorescence with a correction for the spectral sensitivity of the instrument; T is the transmission, %; λ is the wavelength, nm.

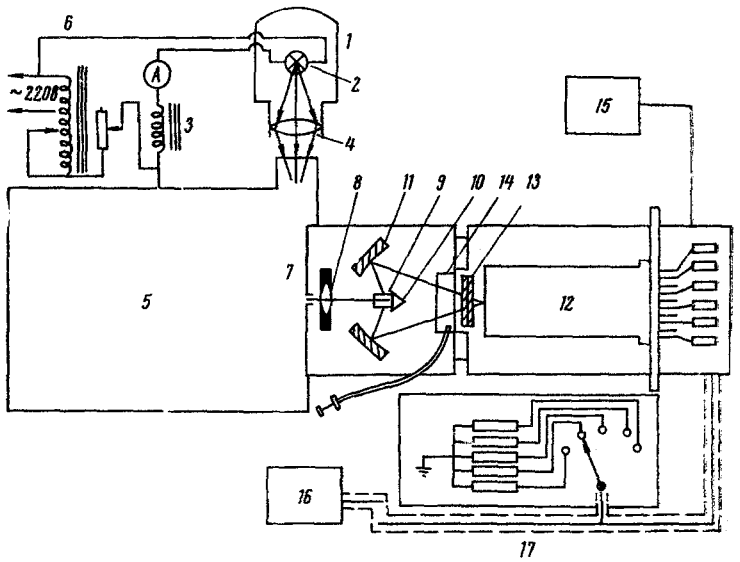

Fig. 2. Diagram of apparatus for determination of serotonin concentration.

bination (OS-11 and SZS-21; see Fig. 1b) or, where necessary, with an interference filter.

Figure 2 shows a block diagram of the apparatus for measurement of the fluorescence excited in solutions by monochromatic radiation. The source of exciting radiation is an OI-17 ultraviolet illuminator for fluorescence microscopes, which has SVD-120 mercury lamp 2, supplied through choke 3, and quartz condenser 4, which focuses the light on the entrance slit of SF-4 monochromator 5. The lamp supply voltage is stabilized by ferroresonance stabilizer 6. After exit slit 7, the monochromatic ultraviolet radiation is focused by quartz condenser 8 onto cuvette 9 containing the sample solution. The 15 × 15 × 6 cuvette is made of crystalline quartz and is mounted in a holder lined with black velvet and fitted with trap 10 for the exciting radiation. In some experiments, we used fused-quartz tubes mounted in a holder of suitable shape. The fluorescence of the solution is focused by two concave mirrors 11 (we used the mirrors from the illuminators of an SF-4 spectrophotometer) onto the photocathode of an FEU-42 photomultiplier 12. In front of the photomultiplier window, there are filters 13 (OS-11 and SZS-21), which isolate the spectral region of the fluorescence, and camera shutter 14. The photomultiplier (800 V) is powered by VS-22 high-voltage rectifier 15. The photomultiplier photocurrent is delivered to LPU-01 tube potentiometric amplifier 16, used in a pH meter. The readings are made directly from the scale of the instrument. The range of measurable concentrations of serotonin (or other substances) is enlarged by having voltage divider 17, consisting of five resistors (10, 5, 2, 1, and 0.5 MΩ), connected between the photomultiplier and the amplifier.

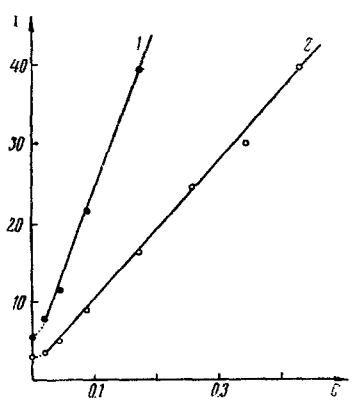

Fig. 3. Plot of the instrument readings I, relative to unity, against the concentration of serotonin C, μg/ml, in aqueous solution (per 1.3 ml of 3 M HCl). The points on the graph are the results of individual measurements. Plots 1 and 2 were obtained with different sensitivity settings of the instrument.

Using the described apparatus, we made a series of determinations of the concentrations of standard serotonin solutions. To 1 ml of standard solution we added 0.3 ml of 12 M HCl. We measured the intensity of the fluorescence excited by radiation of wavelength 296 nm. The results of the measurements are given in Fig. 3. As this figure shows, the method enables us to determine serotonin in a concentration of more than 0.02 μg/ml with an error ±10 to ±2%, depending on the concentration. This is approximately five times more sensitive than the method described in [8].

In conclusion, it should be noted that the apparatus can be used for the assay of other substances, such as histamine or catecholamines, after they have been converted to fluorescent compounds [8]. Since the fluorescence of the latter, however, can be excited by the 365-nm line of a mercury lamp, which can easily be isolated by glass filters, the apparatus described here does not have such pronounced advantages over industrially produced fluorometers. The same applies to the use of the apparatus for determination of

the permeability of tissue barriers by means of fluorescent dyes [9,10].

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