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Identification of substances interfering with the fluorometric determination of brain histamine

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THERE are two principal procedures available for the determination of histamine in biological material: a bioassay method and the fluorometric procedure which is dependent upon the fluorescence of a histamine-*o*-phthalaldehyde (OPT) complex.[1] Both procedures require a preliminary purification of the tissue extract prior to assay. When the fluorometric procedure was introduced, the results obtained indicated good general agreement with the bioassay method, in tissues from various organs and animal species. In 1961, Graham[2] reported that the fluorometric procedure was inadequate for the analysis of liver and plasma in her laboratory. Carlini and Green[3, 4] have since reported that when a rat brain extract is purified by the method of Adam,[5] the bioassay procedure gives values approximately one third of those previously obtained by either bioassay or fluorescence assay. These authors indicate that the previously reported ostensible agreement between the two assay methods is due to the presence of substances other than histamine which give a histamine-like response on bioassay and in the fluorometric method—substances other than histamine which also fluoresce with OPT. In the present work, the substances that form fluorescent compounds with OPT are identified, and a procedure for their separation from brain histamine is described.

Examination of the fluorescence spectrum of authentic histamine, in contrast to "brain histamine" obtained by either a solvent purification procedure,[1] or a chromatographic procedure using Dowex 1-X8[6] as shown in Fig. 1, indicates a spectral shift to the shorter wavelengths. The extent of this shift is considerably greater in the presence of phosphate (as illustrated) than chloride ion (the procedure used by Shore *et al.*[1]).

The histamine assay procedure used is: to 1·0 ml of solution (approximately neutral) add 0·2 ml of 0·5 N NaOH, 0·05 ml 1% OPT solution (in absolute methanol); after 3·5 min add 0·10 ml 2·5 M H_3PO_4; solution is mixed after each addition.[6] The fluorescence is measured at 450 mμ, with activation at 350 mμ (Aminco-Bowman spectrophotofluorometer, wavelength uncalibrated).

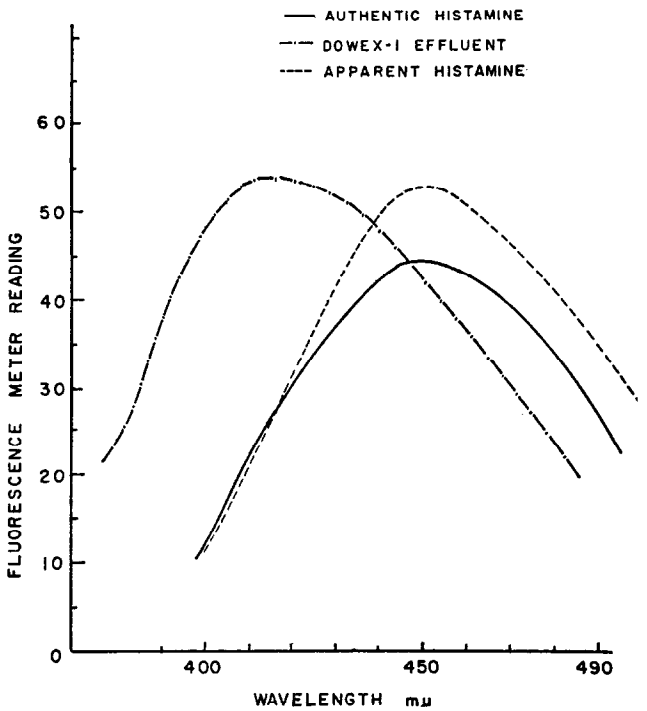

FIG. 1. Fluorescence spectra of authentic histamine; Dowex-1 effluent of guinea pig brain extract and "apparent" histamine after purification with phosphrylated cellulose as described.

A resolution of the substances present in the Dowex-1-purified extract was achieved by the use of phosphorylated cellulose (Cellex P, Bio Rad Laboratories) purified as described previously.[7] A column of the cellulose having a volume of approximately 3 ml, with a height to diameter ratio of 4 to 1, was formed in 0·03 M sodium phosphate buffer, pH 6·5. The Dowex-1-purified extract was adjusted to pH 6·5, applied to the column, and followed by several volumes of buffer and water. The column was eluted with a HCl gradient (0·01 N to 0·20 N). The fluorescence spectrum of "apparent" histamine obtained by this procedure is shown in Fig. 1. Assay of the recovered histamine, by either bioassay or fluorescence procedure, indicated similar values, corresponding to 40–100 ng/g tissue, dependent on the source of brain tissue (i.e. the animal used).

The major contaminating component was eluted after histamine. On the basis of chromatographic behavior, fluorescence spectra, chemical properties, and levels found in tissue, it is concluded that this component is spermidine. Spermidine has not previously been reported to form a fluorescent complex with OPT. The activation and fluorescence spectra of spermidine–OPT complex is shown in Fig. 2; the activation and fluorescence maxima are at 338 and 410 mμ.

The amino acid arginine was also identified in the Dowex-purified brain extract; under the chromatographic conditions described, arginine is not retained by the column. Arginine is known to form a fluorescent complex with OPT, with an activation and fluorescence maximum at 340 and 480 mμ, in alkali.[8] Under the conditions of the histamine assay, where the fluorescence is measured at a pH of approximately 2, the fluorescence of arginine, which is normally measured at an alkaline pH, is slight. The fluorescence contribution of arginine to the total fluorescence of the Dowex-purified brain extract at 450 mμ (histamine fluorescence maximum) was found to be 2% to 10%.

The variation in fluorescence contribution is due to the slow increase in the fluorescence of arginine, with time, in the phosphoric acid system used. In the HCl system[1] this increase was not observed.

The amine agmatine is also known to form a fluorescent compound with OPT under conditions similar to arginine.[8] This amine has a chromatographic behavior very similar to histamine; however, our studies indicate that the concentration of agmatine is very low in brain tissue. Under the conditions of the histamine assay, the fluorescence contribution is similar to, or only slightly above, blank values.

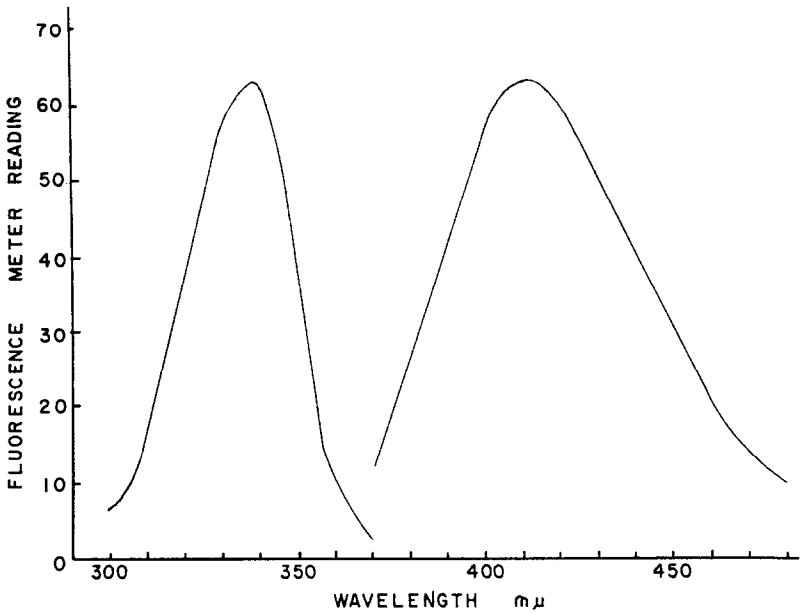

FIG. 2. Activation (peak 338 mμ) and fluorescence spectra (peak 410 mμ) of spermidine, 2·4 μg/ml (histamine assay procedure).

On the basis of these studies in which rat, rabbit, and guinea pig were used, it is concluded that the major interfering substance in the fluorometric estimation of brain histamine is spermidine. As spermidine is present in most biological materials,[9] it probably influences the determination of histamine in most tissues, dependent upon the quantity of spermidine present relative to the histamine concentration. Brain tissue is characterized by the presence of approximately 1,000 times more spermidine than histamine in both the rat and guinea pig brain. Histamine analysis of brain tissue rostral to the quadrigemina of the mature rat, guinea pig, and rabbit (New Zealand white), by this procedure gives mean values (based on five or more determinations) of 76, 57, and 65 ng/g tissue wet weight respectively. Preliminary data with a strain of Dutch rabbits indicates the possible existence of strain differences in brain histamine values. With the newer fluorescent technique the analysis of histamine, spermidine, agmatine, and arginine can be made on the same sample.

Recent experiments demonstrate that the histamine analysis may be made directly by means of the single phosphorylated cellulose column described. The 5% TCA tissue extract, after centrifugation, is diluted with 9 volumes of 0·01 M sodium phosphate buffer, pH 6·0, neutralized with 1 N NaOH to pH 6 and applied to the column. This is followed by approximately 15 ml of the same buffer, 5 ml of water, and 10 ml of 0·01 N HCl. The histamine is quantitatively eluted with 20 to 25 ml of 0·03 N HCl, collected in several tubes for analysis. The column flow rate is not critical and may be completed in about 1 hr. In the analysis of histamine, 0·25 ml of 0·5 N NaOH is used in place of 0·20 ml formerly used to compensate for the additional acid present; 10 μliters of 10^{-2} M EDTA is added to a 1·0-ml sample aliquot before forming the fluorophore.

A column of the size described is adequate for the equivalent of 3 g of brain tissue, or 1 g of those tissues in which the histamine content is greater (liver, kidney, etc.).

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Bureau of Research in Neurology & Psychiatry,
New Jersey Neuropsychiatric Institute,
Princeton, N.J., U.S.A.

LEON T. KREMZNER
CARL C. PFEIFFER

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First studies on foetal organotropism of cephalosporin, streptomycin and rifamycin SV under physiological conditions

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DRUGS exert an action on foetal organism which depends mainly on the stage of pregnancy, on the transplacental passage and on the distribution and localisation in the blood and different foetal organs.

Drug concentration has been extensively investigated in foetal and maternal blood, but scantily at the level of foetal organs (organotropism)[1].

Since 1964,[2–5] we have been carrying out a systematic research on foetal organotropism, by studying drug organotropism under different conditions (physiological, pathological and artificial) and at different times after drug administration.

MATERIAL AND METHODS

Eighty eight Dutch rabbits, weighing 2400 ± 200 g, were used within the period from the 25th to the 30th day of pregnancy. The following drugs were administered to rats which had been fasting for 18 hr: cephalosporin hydrochloride (50 mg/kg i.m.), or streptomycin sulphate (300 mg/kg i.v.), or rifamycin SV Na salt (50 mg/kg i.v.). Animals were killed by bleeding 1/2, 1,2, 4 or 8 hr after streptomycin injection, 1/2, 1, 2, 4, 8 or 14 hr after cephalosporin injection and 1/4, 1/2 or 1 hr after rifamycin SV injection.

The assay of antibiotics was performed by a microbiological method, employing as test-organism *Staph. Curcio* for cephalosporin and rifamycin SV and *Myco. paratubercularis* ATCC 607 for streptomycin. The following organs: kidney, liver, lung, brain, muscle, placenta were aseptically homogenized for 5 min with a M/20 buffer phosphate solution pH 7 (Na_2HPO_4, $12H_2O$ and KH_2PO_4). The homogenates were centrifugated for 10 min at 3000 rev/min. Assays were performed on the supernatants.

RESULTS AND CONCLUSIONS

The results obtained are shown in Figs. 1–3.

1. From a quantitative point of view, blood and organs show, in all three cases an antibiotic activity which is much lower but more lasting in the foetus than in the mother.