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June 8, 1971

REFERENCES

SMITH, A. & HAYASHIDA, K. (1970). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **28**, 261.
SMITH, A., HAYASHIDA, K. & KIM, Y. (1970). *J. Pharm. Pharmac.*, **22**, 645.

The fluorimetric determination of thymoxamine in plasma

Thymoxamine hydrochloride, a specific competitive α-adrenoreceptor blocking drug, and its deacetylated derivative have fluorescent properties which may be exploited for the purposes of recognition and measurement in body fluids and in pharmaceutical formulations. Thymoxamine hydrochloride fluoresces in de-ionized aqueous solution, the pH for maximum fluorescence lying between 7–8. Fluorescence falls more steeply towards alkaline than acid pH and there is another peak at pH 1–1·5 (Fig. 1). Desacetylthymoxamine hydrochloride has the same excitation and emission wavelengths as thymoxamine hydrochloride.

The pK_a values of thymoxamine were found to be about 8·6 and 2·0. Concentrations between 100 ng to 50 μg extracted from water gave a straight line relationship with fluorescence, beyond which quenching occurred. No change in fluorescence of the extract was observed for up to 24 h at room temperature (20°), while the aqueous solution continues to increase in fluorescence for at least this length of time. Benzene with 1·5% isoamylalcohol gave the most consistent recovery of the drug from standard aqueous and plasma solutions, when compared with heptane, chloroform and ethyl acetate. Materials used were:

Thymoxamine hydrochloride 50 mg capsules, solution for intravenous injection 5 mg/ml and powder (Opilon), (William Warner & Co.); desacetylthymoxamine hydrochloride powder, (William Warner & Co.); benzene and isoamylalcohol, Grade A, Analar, (May & Baker Ltd.); sodium hydroxide solution, 1 N in deionized water; and hydrochloric acid, 0·1 N in de-ionized water.

Standard thymoxamine solution was prepared from 10 mg of powder in 10 ml of de-ionized water.

Plasma (2·5 to 4 ml) separated from heparinized blood was extracted by shaking with 1 ml of 1N sodium hydroxide and 12 ml of benzene containing 1·5% isoamylalcohol for 10 min on an automatic shaker. After centrifugation, 10 ml of the supernatant organic layer were added to 1·5 ml of 0·1N hydrochloric acid in another set of stoppered centrifuge tubes for extraction of the drug into the acid phase by shaking for 10 min. After centrifugation, 1·2 ml of the acid phase from the bottom layer was transferred to test tubes and immersed in a boiling water bath for 30 min. The tubes were then cooled to room temperature and the fluorescence of the acid phase measured with an Aminco-Bowman spectrophotofluorimeter at maximum excitation 295 nm and maximum emission 335 nm (uncorrected).

For each subject, duplicate plasma samples were taken at different times before and after administration of the drug. Internal standards were obtained by adding known amounts of thymoxamine to plasma samples together with control blanks which did not contain the drug and these were treated in the same way.

A straight line relationship was obtained with concentrations between 0·2 and 1 μg of drug in plasma and its fluorescence (Fig. 2).

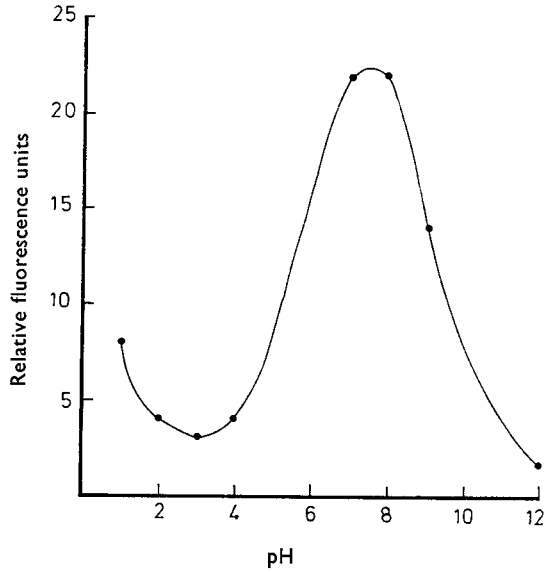

FIG. 1. Relation between fluorescence of a solution of thymoxamine hydrochloride (25 μg/ml in water) and its pH.

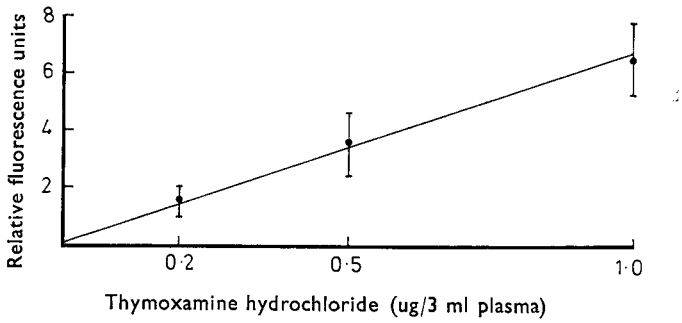

FIG. 2. Relation between concentration of thymoxamine hydrochloride added to plasma, and the fluorescence obtained after extraction. Each point represents a mean ± standard deviation of 16 observations.

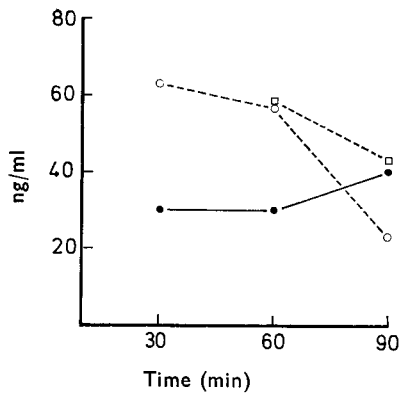

FIG. 3. Plasma levels (ng/ml) obtained at 30, 60 and 90 minutes after administration of thymoxamine hydrochloride 150 and 300 mg to three subjects. □, ○ 150 mg; ● 300 mg.

Four female volunteers (20–30 years) were given 150 mg of thymoxamine in capsule form as a single dose, half an hour after a standard lunch. Blood samples of 15 ml were taken in heparinized tubes before and at 30, 60 and 90 min after ingesting the drug; the procedure was repeated one week later with 300 mg of thymoxamine. In only two of the four subjects were blood concentrations detected after the 150 mg dose, and in these none was found after 300 mg. In one subject, in whom none was found after 150 mg, the drug was detected after 300 mg (Fig. 3).

Two male volunteers (33 years), lying down, were given 0·2 mg/kg of the drug intravenously over 2 min. In one volunteer it was given as a solution of strength 5 mg/ml, and in the other it was diluted in 20 ml of normal saline. Blood samples of 15 ml each were taken in heparinized tubes before and at 2, 7 and 15 min after the end of injection. Plasma concentrations at 2, 7 and 15 min were 140, 83 and 50 ng/ml respectively in the first subject, and 100, 40 and 30 ng/ml in the other.

The fluorescence of thymoxamine is much less than that of deacetylated thymoxamine, although their emission and excitation characteristics are similar. The demonstration that physical and chemical procedures such as boiling and acid hydrolysis increased the fluorescence of thymoxamine in water (Arbab, 1970, unpublished observations) suggests that it is the deacetylated form of thymoxamine which is being extracted and measured in the procedure described in this communication. Deacetylation may well be an important metabolic pathway for thymoxamine, and this extraction method does not distinguish the unchanged drug from its metabolite.

Unfortunately, the calibration curve relating fluorescence to plasma concentration with the extraction procedure described is shallow (Fig. 2), and does not permit accurate determinations of plasma concentrations at the nanogram amounts per ml obtained with therapeutic doses of the drug, but duplicate determinations provide at least a semi-quantitative measurement. The greater intensity of fluorescence at a pH of 7–8 than at the pH of about 1·5 used in this method suggests that the sensitivity of the procedure might be increased if it was modified to permit a final extraction at the alkaline pH.

We thank William Warner & Co. Ltd. for financial support and supplying thymoxamine hydrochloride and its deacetylated derivative. A.G.A. is in receipt of a Colombo Plan Scholarship.

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May 21, 1971