Although the exact nature of the specificity cannot be determined from this observation, both compounds containing more than one asymmetric carbon, it may explain the relatively greater toxicity of yohimbine, which is approximately four times more toxic than corynanthine.

Of greater significance is the fact that corresponding β-carbolines derived from serotonin, 5-methoxytryptamine, and melatonin are all potent MAO inhibitors as well as being serotonin antagonists and capable of disrupting conditioned behavior.[5, 6]

Biological Research Division,
Houston State Psychiatric Institute,
Houston, Texas, U.S.A.

WILLIAM M. MCISAAC
VICENTE ESTEVEZ

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Increased sensitivity in a specific fluorometric method for brain histamine

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THE FLUOROMETRIC determination of tissue histamine by the method of Shore *et al.*[1] has been reported to be inadequate for the analysis of brain histamine because of the presence of interfering substances[2, 3] that are not removed by butanol extraction or by the use of certain ion-exchange columns.[4] Recently, Kremzner and Pfeiffer[5] have shown that the major interfering substance, spermidine, may be separated from histamine by the use of a phosphorylated cellulose column. Their procedure requires elution of histamine from the column with 20–25 ml of 0·03 N HCl. As the concentration of histamine in rat brain is reportedly in the range of 50–76 ng/g,[2, 5] the use of this large elution volume limits the sensitivity of the method, particularly if it is desired to perform regional analysis of histamine in brain. We have modified the Kremzner and Pfeiffer procedure, therefore, so as to allow elution with only 5 ml fluid, thus increasing sensitivity sufficiently so that 0·5 g brain or less may be analyzed, instead of the 3 g required by Kremzner and Pfeiffer.[5]

Phosphorylated cellulose (Cellex-P, Bio Rad Laboratories) was purified as described by Kremzner and Wilson[6] and suspended in 0·03 M phosphate buffer, pH 6·0. The cellulose was then transferred into a chromatographic column (Scientific Glass no. JT-7390, 200 mm length, 6 mm bore) to a height of 40 mm and washed with 10 ml of 0·03 M phosphate buffer, pH 6·0. Histamine (0·1 μg), spermidine (100 μg), or both were applied to the columns in 10 ml of 0·03 M phosphate buffer, pH 6·0. The columns were then washed with 5 ml water and the histamine eluted with 5 ml of 0·2 M NaCl. The eluates were analyzed for histamine and spermidine fluorometrically after condensation with *o*-phthalaldehyde.[1] Recovery of histamine added to the column was 101 per cent. Spermidine could not be detected in the eluates from the columns containing spermidine.

Male Sprague-Dawley rats weighing 200–250 g were used for the determination of brain histamine. Brains were homogenized in 10 volumes of 0·4 N $HClO_4$, centrifuged, and a 5-ml aliquot of the

supernatant fluid was extracted by the procedure of Shore *et al.*[1] The final 0·1 N HCl extract was neutralized to pH 6·0 with 0·1 N NaOH, diluted to 10 ml with 0·03 M phosphate buffer, pH 6·0, and applied to the cellulose column. The column was washed with 5 ml water and the histamine eluted with 5 ml of 0·2 M NaCl. Histamine was then condensed with *o*-phthalaldehyde, as described previously.[1]

Isotope dilution experiments were performed to check the specificity of the method. Several portions of rat brain were extracted by the method of Shore *et al.*,[1] and the final 0·1 N HCl extracts pooled. Histamine dihydrochloride-2-(ring) ^{14}C (specific activity: 1·52 mc/m-mole; Nuclear Chicago Corp.) was added to this acid solution to produce an estimated 10,000 counts/min/μg histamine. A portion of this solution was neutralized to pH 6·0 with 0·1 N NaOH, diluted tenfold with 0·03 M phosphate buffer, pH 6·0, and passed through a Cellex-P column, as described above. A portion of the 0·2 M NaCl eluant from this column was then diluted tenfold with 0·02 M phosphate buffer, pH 6·0, and passed through a second Cellex-P column; elution was again made with 5 ml of 0·2 M NaCl. Aliquots of the original 0·1 N HCl extract and the two column eluates were analyzed fluorometrically. Histamine-^{14}C was counted in a Beckman liquid scintillation counter. The results (Table 1) show that the specific activity of apparent histamine increased considerably upon passage through the first column but remained constant upon passage through the second column, indicating that the column removed interfering substances from the original extract.

TABLE 1. SPECIFIC ACTIVITY OF APPARENT RAT BRAIN HISTAMINE AFTER SERIAL SEPARATIONS

	Specific activity (counts/min/μg)
Butanol extraction and return to 0·1 N HCl	4400
Elution from first Cellex-P column	8200
Elution from second Cellex-P column	8400

See text for details.

TABLE 2. LEVELS OF HISTAMINE IN RAT BRAIN

Tissue	(ng/g $\pm$ S.E.)*
Whole brain	46 $\pm$ 0·03 (10)
Hemispheres	50 $\pm$ 0·03 (11)
Brain stem	116 $\pm$ 0·013 (11)
Cerebellum	31 (3)

* Each value represents mean $\pm$ standard error. Numbers in parentheses indicate number of analyses in each group.

Table 2 shows the results obtained by this method on whole rat brain and on selected parts of brain. The unequal distribution of histamine parallels that reported by Adam[7] in dog brain. As little as 15 ng histamine carried through the procedure gives a reading in the Aminco-Bowman spectrophotofluorometer (activation 360 mμ, fluorescence 450 mμ, uncalibrated) about twice that of the reagent blank.

Dept. of Pharmacology,
University of Texas
Southwestern Medical School,
Dallas, Texas, U.S.A.

M. MEDINA
P. A. SHORE

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Brain amines and brain weights in growing chicks: some normal values and effects of feeding excess dietary L-phenylalanine*

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THE CONCENTRATIONS of serotonin and norepinephrine in the brains of adult chickens have been determined.[1–3] However, the changes in concentration with age are not known for an avian species, although this information is available for several mammalian genera such as rabbit;[4] rabbit, rat, and guinea pig;[5] and cat.[6] This communication provides information on increases in brain amine levels in growing chicks from hatching to 8-week age. In addition, the effect of feeding excess dietary L-phenylalanine on brain amines was studied in chicks, since this amino acid has been shown to depress the brain serotonin level when fed to rats.[7] In the chick, incorporation of L-phenylalanine into the diet causes growth retardation and a variety of physical deficits.[8, 9]

MATERIALS AND METHODS

Three strains of chickens (*Gallus domesticus*) were employed. One strain designated broiler stock (males) was obtained from the Munroe Hatchery, Inc., Joliet, Ill. The other two, white leghorn (females) and a crossbred strain of Rhode Island reds and Plymouth barred rock (males), were from a local source. The normal values were obtained primarily with the broiler stock, and this strain was also fed a diet containing 5% L-phenylalanine. The other two strains were fed 2, 4, or 8% phenylalanine. All animals were maintained under standard poultry-raising conditions with a basal diet consisting of a 19 per cent protein commercial corn-soy ration (Wayne-Allied Mills). Appropriate amounts of L-phenylalanine (Mann) were intimately mixed with the basal diet to obtain the required percentages of this amino acid.

Normal chicks from the broiler stock were killed at 1, 7, 14, 28, or 56 days of age. Those receiving 5% L-phenylalanine were killed at 28 or 56 days. In addition, three chicks which had been on the normal diet for 28 days were placed on 5% L-phenylalanine, and three chicks receiving 5% L-phenylalanine were transferred to the basal ration for 28 days. The white leghorns and crossbred chicks were killed after receiving the L-phenylalanine diets for 28 days. After decapitation the brains were rapidly removed, weighed, frozen on solid carbon dioxide, and subsequently analyzed for their content of serotonin and norepinephrine by the method of Shore and Olin[10] as modified by Mead and Finger.[11] In a few experiments dopamine was also determined by the oxidizing conditions described by McGeer and McGeer.[12] Statistical comparison of results was based on Student's *t* test. Further methodological details are reported in previous publications.[2, 8, 9]

RESULTS AND DISCUSSION

The brain amine values in normal growing chicks of the broiler strain are summarized in Table 1 along with body and brain weights. After the first week of age the brain concentrations of both norepinephrine and serotonin show no further increases. Mammals, which, with the exception of the guinea pig, are born relatively undeveloped compared to the maturational level of the chick at the time of hatching, require several weeks or months to attain constant amine concentrations in the brain. There was no difference in the brain serotonin content among the three strains when compared at 4 weeks of age (Table 2). However, the norepinephrine content of the broiler strain was appreciably higher than those of the other two breeds (Table 2).

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