The fluorescence spectra of 5-HT and 5-HIAA, expressed in arbitrary units of fluorescence, are given in Fig. 1. Rat brain 5-HT and 5-HIAA amounted to 0·600 ± 0·032 μg/g and 0·361 ± 0·015 μg/g, respectively. The results obtained are in agreement with the values of 5-HT found previously by two[8] of us in a large number of animals and with the 5-HIAA values reported in the literature.[6-9]

No reciprocal influence of 5-HT and 5-HIAA were observed in the simultaneous estimations performed in this study. As can be seen from the S.E.'s reported, the reproducibility of the method described is very satisfactory.

In conclusion, the present method is simple and reliable. It offers the possiblity to study 5-HT/5-HIAA ratios in the same sample, and, accordingly, changes in the metabolism of 5-HT under the influence of pharmacological substances.

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The influence of oxotremorine on iron and flavines in mitochondria from rat brain corpus striatum*

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THERE is much evidence to support the importance of corpus striatum in the genesis of static tremor.[1,2] The area of globus pallidus seems to be particularly involved in the development of tremor, since its destruction in Parkinsonians leads to a marked alleviation of symptoms.[3] Globus pallidus is an area of considerable biochemical interest for its high concentration of iron[4] and flavines.[5] Therefore a knowledge of the behavior of these factors during tremor is desirable. We have been able to show in earlier reports that tremor-producing drugs, such as tremorine (1:4-dipyrrolidinobutyne), and oxotremorine (2′-oxo-1:4-dipyrrolidinobutyne), cause a considerable decrease of iron[6] and flavines in whole rat brains[7] and rat corpora striata.[8] The present work was intended to study parallel changes of iron and flavine concentrations under the influence of oxotremorine, and, in view of a possible metabolic meaning of such changes, rat corpus striatum mitochondria were examined instead of whole tissue.

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Wistar albino rats, weighing 250–300 g, were injected intravenously with a small dose (0·25 mg/kg) of oxotremorine (OT, kindly donated by Professor Dr. E. Testa, Lepetit Company, Milano, Italy). This dose was found to be convenient in previous work.[7,8] After the lapse of specified periods the animals were sacrificed and their brains were flushed free of blood *in situ*, then rapidly removed and corpora striata excised. Tissues from several brains were pooled and frozen until use. The mitochondria were separated from supernatants of tissue suspensions in 0·33 M sucrose (after removal of nuclei and cell debris) by centrifugation at 3×10^4 *g* for 1 hr in a refrigerated centrifuge. Non-heme iron in mitochondrial pellets was determined by the method of Foy *et al.*;[9] for determinations of total iron Foy's procedure was carried out with the residue after wet-ashing a part of the particles suspension (corresponding to 10–15 mg fresh mitochondria). Total flavines were estimated by the method of Bessey *et al.*[10] The results obtained with experimental animals were compared to those of controls, receiving physiological saline instead of the drug.

Table 1 shows that OT exerts a biphasic effect on the iron in mitochondria: A drop of total iron occurs first, and reaches a maximum ½–1 hr after injection. Total flavines remain essentially unchanged during this period. In the second phase (1–2 hr after injection) the iron level rapidly recovers, and rises even considerably above the control values, while total flavines become markedly reduced.

TABLE 1. CHANGES OF TOTAL IRON AND FLAVINES IN RAT BRAIN CORPORA STRIATA AFTER INTRAVENOUS OXOTREMORINE (0·25 mg/kg)

Time after injection (min)	Total iron Per cent of control values (number of animals)	Total flavines Per cent of control values (number of animals)
15	77·6* (6)	102·9 (6)
30	76·4* (8)	98·0 (6)
60	101·7 (7)	96·1 (7)
90	121·3* (7)	73·0* (7)
120	142·8* (7)	65·5* (6)

* Differences between experiments and controls are statistically significant at the $P < 0{\cdot}005$ level (*t*-test). Control values were obtained from nine animals and their values are given in Table 2.

A comparison of total and non-heme iron levels, estimated thirty minutes after injection (i.e. at a point of maximum reduction of the iron level), demonstrates that non-heme iron is not affected by the drug (Table 2), which means that the drop of total iron must be due to a decrease of functional iron.

TABLE 2. CHANGES IN TOTAL AND NON-HEME IRON IN MITOCHONDRIA OF RAT *CORPORA STRIATA* 30 min AFTER INTRAVENOUS OXOTREMORINE (0·25 mg/kg)

	Iron μg/mg (wet)	
	Total	Non-heme
Control	79·7 ± 8·8 (8)	14·8 ± 3·9 (8)
Experimental	60·9 ± 3·7 (8)	13·8 ± 4·4 (8)
Relative change (% of control)	−23·5	n.s.

Numbers of determinations are given in parentheses. "n.s." "means" statistically non-significant at the 0·05 level (*t*-test).

From the results reported in the present paper there seems to be no relation between the changes in total iron contents and those of flavines, as a depletion of the first, following intravenous OT precedes depletion of the second, and an uptake of iron apparently occurs during the depletion of flavines.

Further investigation is expected to show the meaning of these results.

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The effect of benzo[a]pyrene derivatives upon drug metabolising enzyme activities

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MANY polycyclic aromatic hydrocarbons are known to induce, in rodents, the synthesis of the so-called "Drug Metabolising Enzymes", a group of apparently related adaptive microsomal enzymes found predominantly in the liver.[1] Recently a number of reports have appeared concerning the induction of "drug metabolising enzymes" by substituted derivatives of aromatic polycyclic hydrocarbons and of related heterocyclic systems.[2–4] It seemed at one time that ability to induce these enzymes was associated with carcinogenicity but recent work, including that quoted above, shows that this is not necessarily so. In view of this and the fact that under a number of circumstances it could be therapeutically desirable to stimulate the activity of these "drug metabolising enzymes", in man and his domestic animals; information upon the effect of substituent groups, in polycyclic aromatic systems, on the induction of adaptive microsomal enzymes is of great interest.

In the course of a systematic investigation of the relationship between substituents and biological properties in benzo[a]pyrene derivatives we studied the effect of a variety of 6-substituted compounds (see formula) upon adaptive microsomal enzymes. Benzo[a]pyrene itself, (R=H), 6-formylbenzo[a]-pyrene, (R=CHO), 6-bromobenzo[a]pyrene (R=Br), 6-chlorobenzo[a]pyrene (R=Cl), 6-methyl-benzo[a]pyrene ($R=CH_3$), 6-hydroxymethyl benzo[a]pyrene ($R=CH_2OH$), benzo[a]pyrene-6-amide ($R=CONH_2$) and benzo[a]pyrene-6-nitrile (R=CN) were examined. We wish to report the effect of these compounds upon the duration of zoxazolamine paralysis and hexobarbital hypnosis in mice. It appears that two different adaptive microsomal enzyme systems are involved in the metabolism of zoxazolamine and hexobarbital, as administration of benzo[a]pyrene increases the rate of zoxazola-mine metabolism, hence shortening the paralysis time, but does not alter hexobarbital metabolism. In contrast to the behaviour of benzo[a]pyrene phenobarbital stimulates both zoxazolamine and