These observations suggest that an endogenous inhibitor is present in the caudate nucleus extract. In an attempt to remove this inhibitor, acetone-dried powders of the caudate nucleus were prepared. The activity was present in the supernatant after the powder had been resuspended in phosphate buffer pH 6.0 (0.1 M). Increasing concentration of this supernatant gave a linear increase in L-DOPA production (Figure 1). The acetone used for the preparation was evaporated and the residue taken up in buffer; this solution significantly inhibited the enzyme. The inhibitory factor in this solution has not yet been identified.

The tyrosine hydroxylase was very unstable both in tissue homogenates and in the supernatant of the acetone powder, losing more than 50% of its activity in 24 h at −18°C. The enzyme in the acetone dried powder was, however, reasonably stable at −70°C. The most satisfactory results were obtained when the enzyme activity in the powder was assayed immediately after the powder was prepared. The tyrosine hydroxylase activity was estimated to be 0.62 ± 0.044 μmol L-DOPA formed/g guinea pig caudate nucleus per hour.

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The effect of fasting on the formation of 4-hydroxy-3-methoxyphenyl-acetic acid (homovanillic acid) and 5-hydroxyindolylacetic acid in the brain of the mouse.

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Fasted rats have an increased concentration of brain 5-hydroxyindolylacetic acid (5-HIAA) which has been attributed to an increase in the synthesis and turnover of 5-hydroxytryptamine (5-HT) (Tagliamonte, Biggio, Vargiu & Gessa, 1973). The possibility that fasting also increases the concentration of homovanillic acid (HVA), one of the acidic metabolites of dopamine, was investigated.

The HVA content of the caudate nuclei and the 5-HIAA content of the forebrain were determined in male albino mice kept in reversed daylight and killed at least two h after the beginning of darkness. The HVA and 5-HIAA were estimated fluorimetrically by the methods of Murphy, Robinson & Sharman (1969) and Ahtee, Sharman & Vogt (1970).

After 20 h fasting, mice showed a higher content of HVA in the caudate nucleus and of 5-HIAA in the forebrain (2.1 ± 0.1 and 0.35 ± 0.01 μg/g respectively) than fed mice (1.3 ± 0.09 and 0.29 ± 0.01 μg/g respectively). Yet, the administration of probenecid produced a smaller increase in the HVA concentration in fasted than in fed mice (33.2% vs 108%). This result could be due to a blockade of the active transport system which removes this metabolite from the brain. If this explanation were correct, the administration of probenecid should affect the content of 5-HIAA in fasted and fed mice in the same way as it affected HVA because both metabolites use a similar transport system. However, after probenecid the increase of 5-HIAA in fasted mice was larger than in fed mice (the opposite result to that found with HVA), confirming that fasted animals have an increase in 5-HT turnover and suggesting a functionally unaltered transport system for HVA and 5-HIAA during fasting.

These results could be explained by the existence of a pool of HVA at a site within the caudate nucleus where the probenecid-sensitive transport is not effective. This hypothetical pool would be increased by fasting and might also be identical with the probenecid-resistant pool suggested by Sharman (1967) to explain the effect of some drugs on the striatal HVA content. Finally, some experiments were carried out to test whether fasting affected the concentration of dihydroxyphenylacetic acid (DOPAC) in the mouse caudate nucleus. No change was found, so the possibility should be considered that there is a subpopulation of dopaminergic neurons, the activity of which leads to the formation of HVA but not DOPAC.

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Some observations on human brain monoamine oxidase

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Johnston (1968) classified monoamine oxidase (MAO) into type A and type B on the basis of studies with the MAO inhibitor clorgyline. Clorgyline is a specific inhibitor of type A MAO (Johnston 1968) and deprenyl of type B MAO (Knoll & Magyar 1972). 5-Hydroxytryptamine (5-HT) is preferentially deaminated by type A MAO (Johnston 1968) and benzylamine by type B MAO (Christmas, Coulson, Maxwell & Riddell, 1972). During a study of the distribution of MAO activity in human brain, using 5-HT and benzylamine as substrates in the assay procedure, we observed that the activity of the enzyme towards 5-HT was relatively greater in the cerebral cortex than in the hypothalamus, caudate, putamen and nucleus accumbens – suggesting a higher proportion of type A MAO in the cortex. By using the specific inhibitors clorgyline and deprenyl we attempted to verify this suggestion on samples of occipital cortex and caudate. The activity of MAO towards 5-HT and benzylamine in the occipital cortex and caudate is presented in Table 1. Tissue samples were homogenized in approximately 20 volumes of 0.05 M phosphate buffer, pH 7.2, with 8 passes of a motorized teflon pestle. MAO activity was assayed by a radiometric technique similar to that described by Robinson, Lovenberg, Keiser & Sjoerdsma (1968).

However, with clorgyline as the specific inhibitor and with tyramine as substrate we were unable to demonstrate a different proportion of type A MAO in six samples of occipital cortex compared with six samples of caudate. Moreover, when inhibitor concentration was plotted against MAO inhibition several of the resulting inhibition curves did not exhibit the expected plateaus and were single sigmoid curves. This result was confirmed with deprenyl. When double sigmoid inhibition curves were obtained with clorgyline as the specific inhibitor they were also obtained with deprenyl. When single sigmoid curves were obtained with clorgyline they were also obtained with deprenyl.

These observations suggest that studies of the characteristics of MAO, using specific inhibitors, on samples of human brain collected and stored under usual conditions may produce results which are difficult to interpret in terms of the type A/type B classification of the enzyme.

Table 1 MAO activity in human cortex and caudate with 5-HT and benzylamine as substrates
Results expressed as n mole product mg protein^{-1} h^{-1} (mean ± s.d.)

	5-HT	*Benzylamine*	*Benzylamine/5-HT*
Occipital cortex ($n = 10$)	56 ± 16	46 ± 11	0.8
Caudate ($n = 10$)	47 ± 6	86 ± 15	1.8

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