presence of substrate protects monoamine oxidase from inhibition by phenethylhydrazine, and the kinetics of benzylamine oxidation in the presence of the inhibitor can be described by a kinetic mechanism involving two competitive reactions.

These results suggest that the oxidation of phenethylhydrazine by monoamine oxidase and the inhibition of the enzyme may be interdependent phenomena.

Studies on the kinetic reaction pathway of phenethylhydrazine oxidation indicate that this monoamine oxidase-catalysed reaction may follow a different kinetic mechanism from the oxidation of more normal substrates. The kinetics are consistent with a mechanism in which ternary complexes are formed and in which no kinetically significant quantities of the free modified enzyme are formed. A kinetic reaction pathway in which the first product does not readily dissociate from the enzyme before the second substrate (oxygen) is bound will fit the data. This would be in accord with the formation of an enzyme-bound hydrazone, which, being resistant to hydrolysis, would not easily dissociate from the enzyme.

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Monoamine Oxidase: Multiple Forms and Selective Inhibitors

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Mitochondrial monoamine oxidase [monoamine-oxygen oxidoreductase (deaminating), EC 1.4.3.4] from various human, rat and bovine organs has been separated by electrophoresis into constituent isoenzymes (Youdim & Sandler, 1967; Kim & D'Iorio, 1968; Collins, Youdim & Sandler, 1968; Youdim, Collins & Sandler, 1969; Gomes, Igaue, Klopfer & Yasunobu, 1969; Gorkin, 1969; Sierens & D'Iorio, 1970). Purified enzyme from human and rat liver mitochondria, for example, exists in five enzymically active forms (Youdim, Collins & Sandler, 1970) whereas that from the brain of both species separates electrophoretically into four active bands (Youdim *et al.* 1969; Collins, Sandler, Williams & Youdim, 1970). The isoenzymes exhibit different physicochemical properties: thermal stability, pH optimum and substrate and inhibitor specificity all vary, sometimes in different regions of the same organ.

Monoamine oxidase inhibitors have been widely used in the treatment of depressive illness, and it has been tacitly accepted that their beneficial effect stems from inhibition of monoamine oxidase alone and the resulting elevation of monoamine concentrations in the central nervous system (Pletscher, Gey & Burkard, 1966). There had previously been no convincing explanation as to why some drugs inhibiting the enzyme are effective in the treatment of depression and others are not (Davis, 1965; Hendley & Snyder, 1968). It would now seem reasonable to suggest that the answer lies in terms of differential inhibition of monoamine oxidase isoenzymes. This hypothesis brings in its train an important corollary: if it were to prove to be correct, it follows that the synthesis of specific inhibitors tailored to an individual isoenzyme band at a particular anatomical site should be within our grasp. Implicit in this argument is the conclusion that the therapeutic effectiveness of monoamine oxidase inhibitors relies on a localized accumulation of a particular amine substrate at a specific site in the brain. The pinpointing of this site is one of the eventual aims of our study. Such is the strategy: this communication outlines some of the tactics.

Most of our efforts in the complex field of inhibitor studies have been concentrated on two organs, liver and brain. The inhibitory effect of iproniazid, pheniprazine, pargyline, harmaline and clorgyline (M&B 9302) *in vitro* on four of the five forms of rat liver monoamine oxidase was measured with four

different substrates, kynuramine, tyramine, tryptamine and dopamine. Wide variations existed in the sensitivity of the isoenzymes to the different drugs and the differential effect depended on the substrate employed. With kynuramine isoenzyme-4 was more resistant to the inhibitory action of iproniazid, pheniprazine, harmaline and clorgyline than were the other isoenzyme forms. However, when tyramine was employed as substrate the pattern was somewhat different; isoenzyme-4 was resistant to inhibition by iproniazid, pargyline and harmaline (Sandler, Collins & Youdim, 1970).

These data have to some extent been paralleled by studies *in vivo* in which clorgyline, tranlycypromine or pargyline were given to rats 2 h before they were killed. Hepatic isoenzymes were prepared and their activities compared with those from untreated animals. Isoenzyme-4 appeared to be more resistant to inhibition by all three drugs when tyramine was used as substrate compared with kynuramine (Sandler *et al.* 1970). It therefore appears that differences in inhibitor affinity *in vitro* may reflect variations *in vivo* and are not simply a consequence of the preparative procedure.

We had shown earlier that the substrate specificities of brain and liver monoamine oxidase isoenzymes from human or rat differ (Collins *et al.* 1968, 1970; Youdim *et al.* 1969, 1970). For example, rat and human liver isoenzyme-1 deaminates tyramine about 20 times as rapidly as isoenzyme-4 does; isoenzyme-1 is particularly sensitive to tranlycypromine and pargyline. This finding may explain why some monoamine oxidase inhibitors induce hypertensive crises with tyramine more readily than others (Blackwell & Marley, 1969).

To suggest a possible biochemical basis for a tranlycypromine-cheese interaction from such data is relatively straightforward. The problem of the differing abilities of monoamine oxidase inhibitors to alleviate depression is not as clear-cut, if one is to judge from the results of our preliminary experiments. Monoamine oxidase inhibitors are useful for the treatment of terminal depression in geriatric subjects. B. Bevan-Jones, G. G. S. Collins, C. M. B. Pare, M. Sandler & M. B. H. Youdim (unpublished work) have recently been able to investigate brains obtained at autopsy from such patients treated with tranlycypromine, clorgyline or isocarboxazid and from untreated controls from the same hospital, whose enzyme activity and distribution did not differ from previously investigated (Collins *et al.* 1970) non-depression brains. In each group whole mitochondrial monoamine oxidase activity was measured in cerebral cortex, cerebellum, centrum ovale, basal ganglia and pineal body with kynuramine, dopamine, tyramine and tryptamine as substrates. After tranlycypromine the oxidation of tryptamine was inhibited less than that of the

other substrates, whereas with isocarboxazid dopamine oxidation was least affected; in the pineal body, in fact, the rate of dopamine oxidation was similar to that of untreated brain, whereas oxidation of tyramine was completely blocked. After clorgyline a complex pattern of enzyme inhibition was seen: in the pineal body, basal ganglia and centrum ovale oxidation of tryptamine was unaffected, whereas inhibition of enzyme from the cerebral cortex was greater than 50%; similarly dopamine oxidation was normal in the cerebral cortex but decreased to almost half in the pineal body.

Evidence has been obtained *in vitro* suggesting that the monoamine oxidase isoenzymes of human brain do indeed possess differing sensitivities to clorgyline (Collins *et al.* 1970). Appropriate experiments *in vivo* await analysis, although a number of such observations of the effect of inhibitors on the monoamine oxidase isoenzymes of rat brain have been made (Youdim *et al.* 1969). The four isoenzymes prepared from human brain as a whole possess markedly differing substrate specificities. Further, the activity of a single band differs greatly between different brain areas (Collins *et al.* 1970). It seems probable that inhibitor studies will show the situation to be even more complex than at present. The physiological significance of the multiple forms of monoamine oxidase has yet to be elucidated. Its substrates include the catecholamines, 5-hydroxytryptamine, tyramine, octopamine and tryptamine; it seems possible that a particular isoenzyme in a specific anatomical site may be predominantly responsible for the inactivation of a particular monoamine. Thus the human brain isoenzyme-4 possesses the highest substrate preference for dopamine, and this substrate specificity, of all areas of the brain examined, is highest in the basal ganglia (Collins *et al.* 1970). This area of the brain is known to be particularly rich in dopamine. It is difficult to believe that such a relationship is without functional significance.

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Monoamine Oxidase Inhibitors and Brain Monoamines in Clinical Conditions

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The amine hypothesis of depression states that at least some cases of endogenous depression are caused by a depletion of certain monoamines in the brain. The hypothesis is supported by the finding that all drugs that elevate mood increase the brain concentrations of free monoamines, such as noradrenaline, whereas drugs that depress mood decrease these concentrations (Schanberg, Schildkraut & Kopin, 1967).

By their enzyme-inhibitory action the monoamine oxidase inhibitors increase the concentrations of monoamines in the brain and can reverse the depressive-like behaviour of animals resulting from amine-depleting drugs such as reserpine. The monoamine oxidase inhibitors were the first group of antidepressants (as distinct from stimulants) to be introduced into psychiatry, and despite adverse publicity are still considered by most experienced psychiatrists to be extremely useful, and indeed essential, drugs, especially for certain cases of depression.

Effect of monoamine oxidase inhibitors on brain monoamines. Measurement of the amount of inhibition of monoamine oxidase by the use of 5-hydroxytryptamine-tolerance tests and changes in the urinary excretion of 5-hydroxyindol-3-ylacetic acid or tyramine have lost favour as being too indirect. What is wanted is a measure of the change in brain amine concentrations brought about by the drug and, if possible, the change in amine activity.

Following on the work of Joyce (1962), we have estimated the concentrations of 5-hydroxytryptamine, noradrenaline and dopamine in the brain stem, hypothalamus and caudate nucleus in

patients who had died of various terminal illnesses and who had been receiving monoamine oxidase inhibitors (MacLean, Nicholson, Pare & Stacey, 1965; B. B. Jones, W. J. Nicholson, C. M. B. Pare, K. Price & R. S. Stacey, unpublished work). The essential findings were that all monoamine oxidase inhibitors had a similar pharmacological effect, as measured by the change in brain amines, provided they were given in doses that were clinically equipotent. Secondly, the maximum concentration occurred at about 2-3 weeks and at a time when a clinical effect would be expected to occur, but thereafter tended to plateau off. Thirdly, although the distribution of individual concentrations are unimodal, there was a wide variation, some patients showing only a small increase in amine concentration.

These findings are interpreted as being very suggestive of the antidepressant effect of the monoamine oxidase inhibitors being mediated through their enzyme-inhibitory properties. The fact that some depressed patients do not respond to the drug may be due in part to some of them needing a larger dose, and Akindele, Evans & Oswald (1970) have shown a correlation between clinical improvement and disappearance of R. E. M. (rapid-eye-movement) sleep, which in certain patients only occurs with large doses of a monoamine oxidase inhibitor. On the other hand, it has been suggested that there may be at least two genetically distinct types of depression, one responding to the monoamine oxidase inhibitors and the other to tricyclic antidepressants, the response to the drugs tending to breed true in the same family (Pare, Rees & Sainsbury, 1962; Pare, 1970).

Brain amines in depression. Studies of brain amines in patients who commit suicide have been somewhat disappointing (Shaw, Camps & Eccleston, 1967; Bourne *et al.* 1968; Pare *et al.* 1969). They showed no evidence whatsoever of a deficiency of noradrenaline or dopamine, but a possible depletion of 5-hydroxytryptamine. These studies measured total brain amines and, of course, do not necessarily reflect the amount of physiologically active amines. These might best be measured by a determination of their metabolites, and indeed there appeared to be a decrease in the amount of 5-hydroxyindol-3-ylacetic acid in suicides compared with controls (Bourne *et al.* 1968; Pare, Yeung, Price & Stacey, 1969), but a study of catecholamine metabolites was impracticable. The suggestion of decreased amounts of 5-hydroxytryptamine and its major metabolites in these studies is, however, made more significant by the finding by Ashcroft *et al.* (1966) and Denker, Malm, Roos & Werdinius (1966) of decreased amounts of 5-hydroxyindol-3-ylacetic acid in the cerebrospinal fluid of depressed patients, and preliminary studies on the cerebrospinal fluid in patients