Although no molecular orbital calculations have been made, a presumptive assignment of the coupling constants of Table 1 may be made on the basis of the directive influence of substituent groups[8] and on the work of Stone and Waters with substituted phenols.[3] This leads to the tentative identification of H′ = H5, H″ = H3, H‴ = H6 (or H4), and H⁗ = H4 (or H6).

By themselves the present data do not support a biological role for the free radical forms of salicylates. Nevertheless, it is germane that many other substituted phenols can be oxidized in a similar way.[3, 6] Hence one may conclude that pharmacological behaviour cannot be determined *solely* by the presence of free radical intermediates, in any case. Substances that can be oxidized to form free radicals at similar redox potentials could have their biological effects modified by other molecular properties, especially those affecting penetration and distribution within cells.

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## The effect of various psychotropic drugs on the activity of tyrosinase

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ONE of the unusual side effects associated with the therapeutic use of the phenothiazine tranquilizers is the production of a Parkinsonian syndrome.[1–5] Little, however, is understood concerning the biochemical mechanism by which the phenothiazines produce the disturbance. One of the most striking biochemical lesions seen with the natural Parkinson's disease is the depigmentation of the substantia nigra. Marsden[6] has demonstrated that the pigment of the substantia nigra probably arises from the action of a polyphenolase, most likely tyrosinase. It thus appeared that one might gain insight into a possible mechanism whereby the phenothiazines, such as chlorpromazine, produce this syndrome by studying the effects of these drugs on tyrosinase activity. Since the ability to produce the neurological disturbance is related to the psychotropic activity or potency of the compounds,[7, 8] it appeared of interest to examine, also, the effects of other psychotropic drugs, such as the monoamine oxidase (MAO) inhibitors.

## METHOD

Tyrosinase activity was measured spectrophotometrically by a Beckman DK-2 spectrophotometer. The reaction was followed by the change in optical density at 310 mμ. Mushroom tyrosinase (Sigma pfs grade) served as the enzyme. The substrates, enzyme, and all additions were put into solution with 0·1 M $Na_2HPO_4$–$NaH_2PO_4$ buffer, pH 6·8. The working solution of tyrosinase contained 0·1 mg of the enzyme/ml and 0·1 ml was used in the assay. The final concentration of the substrates ranged from $0{\cdot}25 \times 10^{-3}$ M to $1{\cdot}0 \times 10^{-3}$ M. The Michaelis constants ($K_m$) for 3,4-dihydroxyphenylalanine (dopa), 3,4-dihydroxyphenethylamine (dopamine), norepinephrine, and epinephrine were determined from Lineweaver-Burk double reciprocal plots.

When the psychotropic drugs were studied, dopa ($1 \cdot 0 \times 10^{-3}$ M) served as the substrate for the reaction, and the drugs were added to make a final concentration of $1 \times 10^{-3}$ M or $1 \times 10^{-4}$ M in the assay mixture. Buffer was added to adjust the final volume to a total of 3·0 ml. The reactions were carried out at room temperature. Enzyme activity was expressed as the change in extinction per sec.

## RESULTS AND DISCUSSION

Table 1 contains the $K_m$ values for the action of tyrosinase on dopa, norepinephrine, epinephrine, and dopamine. The enzyme appeared to have a greater affinity for dopamine and dopa, which show

TABLE 1. $K_m$ VALUES FOR THE ACTION OF TYROSINASE ON DOPA AND THE CATECHOLAMINES

| Substrate | $K_m$* |
|---|---|
| Dopamine | 0·50 |
| Dopa | 0·59 |
| Epinephrine | 0·71 |
| Norepinephrine | 1·14 |

*Each figure represents the average of 3 different determinations.

the lowest $K_m$ values. The greatest affinity appeared to be for dopamine. Although the $K_m$ values are only slightly different for dopamine and dopa, they were consistently so.

Table 2 presents the results obtained with two phenothiazines and several MAO inhibitors. The

TABLE 2. EFFECT OF PSYCHOTROPIC DRUGS ON TYROSINASE ACTIVITY

| Substance | Dopa conc. (M) | Inhibitor conc. (M) | Enzyme activity ($E310 \times sec^{-1}$) | Inhibition (%) |
|---|---|---|---|---|
| Control | $1 \times 10^{-3}$ | | $10 \cdot 0 \times 10^{-3}$ | |
| Phenelzine (Nardil) | $1 \times 10^{-3}$ | $1 \times 10^{-3}$ | $1 \cdot 3 \times 10^{-3}$ | 87·0 |
| Phenelzine | $1 \times 10^{-3}$ | $1 \times 10^{-4}$ | $6 \cdot 7 \times 10^{-3}$ | 33·0 |
| Phenelzine (preincubated)* | $1 \times 10^{-3}$ | $1 \times 10^{-4}$ | $5 \cdot 3 \times 10^{-3}$ | 47·0 |
| Iproniazid (Marsilid) | $1 \times 10^{-3}$ | $1 \times 10^{-3}$ | $12 \cdot 0 \times 10^{-3}$ | 0·0 |
| Iproniazid (preincubated)* | $1 \times 10^{-3}$ | $1 \times 10^{-3}$ | $11 \cdot 0 \times 10^{-3}$ | 0·0 |
| Chlorpromazine (Thorazine) | $1 \times 10^{-3}$ | $1 \times 10^{-4}$ | $10 \cdot 0 \times 10^{-3}$ | 0·0 |
| Chlorpromazine (preincubated) | $1 \times 10^{-3}$ | $1 \times 10^{-4}$ | $10 \cdot 0 \times 10^{-3}$ | 0·0 |
| Control | $1 \times 10^{-3}$ | | $18 \cdot 0 \times 10^{-3}$ | |
| Pheniprazine (Catron) | $1 \times 10^{-3}$ | $1 \times 10^{-4}$ | $18 \cdot 0 \times 10^{-3}$ | 0·0 |
| Triflupromazine (Vesprin) | $1 \times 10^{-3}$ | $1 \times 10^{-4}$ | $19 \cdot 0 \times 10^{-3}$ | 0·0 |

* The drugs were preincubated with the enzyme for 10 min prior to the assay.

only compound that exhibited an inhibitory action on the enzyme was phenelzine, a MAO inhibitor. Neither of the phenothiazine derivatives was inhibitory at the concentrations studied. The concentrations used in these experiments were higher than those that would be anticipated to be present in the brain after the administration of the drugs. Iproniazid and pheniprazine, also MAO inhibitors, had no influence on the tyrosinase reaction. The inhibition produced by phenelzine was of the noncompetitive type.

Since phenelzine inhibited the reaction and iproniazid did not, it was at first thought that the inhibition may have been dependent upon the free hydrazine group. To examine this possibility, pheniprazine, which also contains a free hydrazine group, was examined for its effects on the enzyme. No inhibition was exhibited by this compound. The inhibition seen with phenelzine was probably nonspecific.

The phenothiazine compounds, which were thought possibly to inhibit the tyrosinase reaction, had no influence on the enzyme. It must be kept in mind that the enzyme preparation used in these studies was a plant enzyme that may exhibit different properties toward the drugs than the enzyme

of the substantia nigra. However, unless there is a marked difference, the present study would indicate that the phenothiazines do not act upon tyrosinase. Since only phenelzine of the MAO inhibitors had any effect on the enzyme, it would appear that there is little significance to the inhibition observed with it.

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# Cholesterol as a presumed metabolite of digoxin and digitoxin*

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GVOZDJAK *et al.* have reported that daily administration of digitoxin to rats for a week results in a definite gradual increase of adrenal cholesterol.[1] This was attributed to the possible conversion of digitoxin to cholesterol. However, the experimental data did not allow a direct demonstration of this conclusion, nor could the possibility of this conversion occurring elsewhere with transport to the adrenal be excluded. The present study was therefore undertaken to test this hypothesis with more direct evidence. Both *in vivo* and *in vitro* systems were used to study the possible conversion of both tritium-labeled glycosides, digitoxin and digoxin, to cholesterol.

## METHODS

*In vivo*

Adult male Sprague-Dawley rats, 150–200 g, were starved overnight prior to use. Random-labeled $^3$H-digoxin (55·8 $\mu$c/mg) or $^3$H-digitoxin (23·3 $\mu$c/mg) were injected i.p. in 47·5% ethanol. The $^3$H-digoxin had a radiochemical purity of 98·49 ± 0·05%.† Unfortunately, the $^3$H-digitoxin was subject to marked degradation after its preparation. Most of this conversion was to the *bis*-digitoxoside. However, paper chromatography revealed that at least 3/4 of the material on the strips was digitoxin and its digitoxosides, 2/3 of this being digitoxin. Although impure, the material therefore still sufficed for the purposes of the present study. These purities were determined by radiochemical analysis following quantitative extraction from paper chromatograms.[2, 3]

Six hr after injection, each animal was killed by decapitation and the blood collected in a heparinized beaker. Both adrenal glands and the liver were rapidly removed and placed in ice-cold 0·9% NaCl. After weighing, the adrenal glands were homogenized in 2 ml of ice-cold 0·9% NaCl and the liver in 5 ml of the saline solution. Plasma was obtained from 1,000 *g* blood centrifugation for 10 min.

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† Expressed as mean ± standard error of 3 chromatographic assays.