MOLECULAR PHARMACOLOGY, 14, 633-643

# Oxidative Pathways for Catecholamines in the Genesis of Neuromelanin and Cytotoxic Quinones

DOYLE G. GRAHAM

*Department of Pathology, Duke University Medical Center, Durham, North Carolina 27710*

(Received December 15, 1977)
(Accepted February 27, 1978)

SUMMARY

GRAHAM, DOYLE G. (1978) Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol. Pharmacol.*, 14, 633-643

The autoxidation, periodate oxidation, and tyrosinase-mediated oxidation of 6-hydroxydopamine, dopamine, norepinephrine, and epinephrine were studied by absorption spectroscopy. Autoxidation and tyrosinase-mediated oxidation of the three catecholamines resulted in dopachrome analogues—aminochrome from dopamine, noradrenochrome from norepinephrine, and adrenochrome from epinephrine—without evidence for the expected intermediates, the *o*-quinones and the corresponding leukochromes. The use of periodate as an oxidant, on the other hand, allowed visualization of the *o*-quinone intermediates and the subsequent conversion to the dopachrome analogues. Cyclization of the *o*-quinones appeared to occur in the order epinephrine > norepinephrine > dopamine, while the rate of autoxidation occurred in the reverse order. The oxidation of 6-hydroxydopamine to its *p*-quinone was visualized under all three oxidizing conditions. However, the oxidation of 6-hydroxydopamine by periodate gave evidence for a transient intermediate, the *o*-quinone, which rapidly tautomerized to the *p*-quinone. The *p*-quinone product of 6-hydroxydopamine was seen to undergo cyclization to aminochrome, with subsequent polymerization.

## INTRODUCTION

Neuromelanin, a unique melanin deposited within the cytoplasm of catecholamine neurons, is a complex polymer bound to lipofuscin granules. Thus it is unlike eumelanin or phenomelanin, which are synthesized within the melanosomes of melanocytes. Several observations on the genesis and composition of neuromelanin support the concept that catabolic pathways exist for catecholamines other than oxidative deamination (monoamine oxidase) and *O*-methylation (catechol *O*-methyltransferase). The histochemical studies of Barden (1), demonstrating that neuromelanin deposited in the substantia nigra and locus ceruleus is generated from polymerization of oxidized products of dopamine and norepinephrine, have been confirmed and extended by Rodgers and Curzon (2). These authors found that dopa, dopamine, norepinephrine, epinephrine, and 5-hydroxytrypamine can serve as substrates for neuromelanin synthesis. Furthermore, the observation that heat denaturation does not destroy the capacity of brain homogenates to promote neuromelanin deposition suggests that the oxidation of neuromelanin precursors proceeds largely through autoxidation and that the role of protein or lipofuscin is to serve as a nidus for polymerization.

Proposals for these oxidative pathways

This investigation was supported by Grant CA 19013 from the National Cancer Institute. This paper is dedicated to the memory of Dr. Thomas D. Kinney, with continuing gratitude for his persistent support.



0026-895X/78/0144-0633$02.00/0

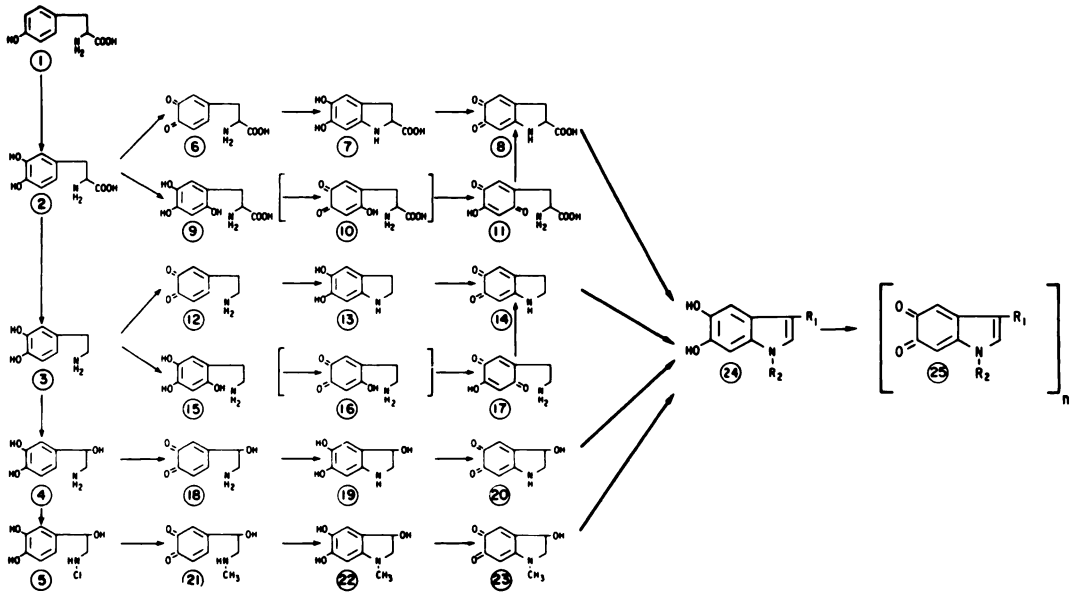

FIG. 1. *Proposed oxidative pathways for dopa and catecholamines*

**1**, tyrosine; **2**, dopa; **3**, dopamine; **4**, norepinephrine; **5**, epinephrine; **6**, dopaquinone; **7**, leukodopachrome; **8**, dopachrome; **9**, topa; **10**, *o*-quinone of topa; **11**, *p*-quinone of topa; **12**, dopamine *o*-quinone; **13**, leukoaminochrome; **14**, aminochrome; **15**, 6-hydroxydopamine; **16**, *o*-quinone of 6-hydroxydopamine; **17**, *p*-quinone of 6-hydroxydopamine; **18**, norepinephrine *o*-quinone; **19**, leukonoradrenochrome; **20**, noradrenochrome; **21**, epinephrine *o*-quinone; **22**, leukoadrenochrome; **23**, adrenochrome; **24**, dihydroxyindole (and derivatives); **25**, indole quinone (and derivatives). *n* refers to the likelihood that this species, perhaps along with other quinones in these pathways, polymerizes to form the various melanins.

can be formulated by analogy with those given for the tyrosinase-mediated oxidation of dopa (3). In addition, the increasing importance attached to the toxicity of 2,4,5-trihydroxyphenylethylamine (6-hydroxydopamine) for catecholamine neurons (4, 5) points to ring hydroxylation as a potentially important metabolic pathway for dopamine, whether this reaction is enzyme-catalyzed or results for autoxidation (6).

The pathways presented in Fig. 1 are based on a variety of studies published in the past 50 years. The sequence from dopa, **2**, to dopachrome, **8**, given by **2**, **6**, **7**, **8**, was described by Raper in the 1920s (7), while the route through topa,[1] **9**, has been proposed only recently as **2**, **9**, **11**, **8**, with the *o*-quinone, **10**, observed only as a transient species when topa, **9**, was oxidized with periodate (3). The observations of Scheulen *et al.* (8) suggested that dopamine as well as dopa could be oxidized by microsomes to quinones that exhibited irreversible binding to proteins. Tse and coworkers have provided evidence for the existence of the sequence **3**, **12**, **13**, **14**, pointing out in addition that the *o*-quinone of dopamine, **12**, is more likely to react with available external nucleophiles (e.g., cysteinyl residues) than to undergo internal cyclization to leukoaminochrome, **13** (9). The genesis of 6-hydroxydopamine, **15**, *in vivo* could result from 6-hydroxylation of dopamine, **3**, as shown or, as suggested by Senoh *et al.* (6), via a nucleophilic attack by water on the *o*-quinone, **12**. As noted by Tse *et al.* (9), however, the latter reaction occurs far too slowly to compete with intracyclization to **13** or with reaction with sulfhydryl groups. Alternatively, 6-hydroxydopamine, **15**, may be produced from decarboxylation of topa, **9** (10, 11).

The oxidation pathway for 6-hydroxydopamine, **15**, has been a matter of some dispute, with Senoh and Witkop (12) and later Saner and Thoenen (13) postulating that oxidation of **15** to the *p*-quinone, **17**, is

[1] The abbreviation used is: topa, 2,4,5-trihydroxyphenylalanine (6-hydroxydopa).

followed by cyclization through attack of the amino nitrogen on the C=C bond to form trihydroxyindoline rather than by attack on the adjacent carbonyl group, which would yield instead aminochrome **14**. The latter pathway, **15**, **17**, **14**, is supported by the observations of Harley-Mason (14) and later authors (15–19), who in addition have established the subsequent formation of 5,6-dihydroxyindole, **24** ($R_1 = R_2 = H$) (14), and the indole quinone **25** ($R_1 = R_2 = H$, $n = 1$), termed by some aminochrome II (15, 19). The existence of the *o*-quinone product, **16**, of 6-hydroxydopamine, **15**, has been postulated by Heikkila *et al.* (20), but the paper by Wehrli *et al.* (21) can be interpreted to reveal that what the former paper called the *p*-quinone is the bromide salt of the protonated form of **17**, and what is termed the *o*-quinone, **16**, is instead the dipolar ion of **17**.

The existence of direct oxidation pathways for norepinephrine, **4**, to noradrenochrome, **20**, and of epinephrine, **5**, to adrenochrome, **23**, were implied in the work by Hochstein and Cohen (22). The sequence **4**, **18**, **19** was proposed by Maguire *et al.* (23) to account for the covalent binding of [$^3$H]norepinephrine to particulate fractions of cells. Similarly, electrochemical studies suggested that the oxidation pathway of epinephrine, **5**, to adrenochrome, **23**, could be written as **5**, **21**, **22**, **23** (24). This sequence was extended to include 3,-5,6-trihydroxy-1-methylindole, **24** ($R_1$ = OH, $R_2 = CH_3$), and the corresponding indole quinone, **25** ($R_1 = H$, $R_2 = CH_3$, $n = 1$), by Harrison *et al.* (25). It is noteworthy that this sequence was suggested by Bu'Lock and Harley-Mason in 1951 (26).

For the sake of simplicity, the formulae for the aminochromes **8**, **14**, **20**, and **23** have been given as the 2,3-dihydroindole 5,6-quinones, and that of **25**, as the indole 5,6-quinones. In aqueous solutions at neutral pH the predominant forms would be the dipolar ions of the iminoquinone tautomers given in Fig. 2 as **28** and **31** and the corresponding anions resulting from dissociation of a proton from the imino nitrogen (19).

In the present investigation the autoxidations of 6-hydroxydopamine, **15**, dopamine, **3**, norepinephrine, **4**, and epinephrine, **5**, have been studied by absorption spectroscopy. The quinone products have been compared with those generated through the action of polyphenol oxidase (tyrosinase, EC 1.10.3.1) and sodium periodate. Because of the probability that catechols like 1,2-glycols are oxidized by periodate via cyclic periodate ester intermediates prior to cleavage by water to *o*-benzoquinones (3, 27), the use of this oxidant held the promise of establishing whether *o*-quinones such as **12**, **16**, **18**, and **21** are intermediates in these oxidative pathways. The genesis of these quinones was studied with the use of rapid repetitive scanning of the ultraviolet and visible spectra at various temperatures.

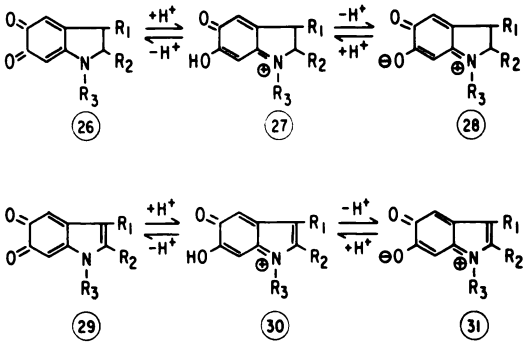


FIG. 2. *Tautomeric rearrangements of 2,3-dihydroindole 5,6-quinones,* **26**, *to the dipolar ionic iminoquinones,* **28**, *and of the indole 5,6-quinones,* **29**, *to the iminoquinone dipolar ions,* **31**, *shown as these would occur in aqueous solutions*

$R_1$ = H or OH; $R_2$ = H or COOH; $R_3$ = H, $CH_3$, $C_3H_7$, etc.

The number of absorbing species in solution was evaluated from these spectra utilizing the matrix analysis of Coleman *et al.* (28). This is an empirical graphical analysis based on simple matrix theory and advantageously applied to reactions with complex equilibria or, as reported here, to kinetic studies. Absorption spectra, taken after various times of reaction, yield multiple values for $A_{ij}$, where $A$ is absorbance, $i$ is the wavelength, and $j$ is the tracing of the spectrum. With one absorbing species in solution, plots of $A_{mj}$ vs. $A_{ij}$, where $i \neq m$ and $m = \lambda_{max}$, yield a family of straight lines passing through the origin. With two absorbing species in solution, linear plots passing through the origin are obtained when $A_{mj}/A_{nj}$ is plotted against $A_{ij}/A_{nj}$,

where $i \neq m$ or $n$ and $n$ = any single wavelength other than $m$, the $\lambda_{max}$. Reactions in which three absorbing species are evident in solution give a family of straight lines not passing through the origin when the quantity $(A_{mx}A_{iy} - A_{my}A_{ix})/(A_{mx}A_{iz} - A_{mz}A_{ix})$ is plotted against $(A_{mx}A_{ij} - A_{mj}A_{ix})/(A_{mx}A_{iz} - A_{mz}A_{ix})$, with $i \neq m$ and $j \neq x, y$, or $z$ ($x$, $y$, and $z$ are three arbitrarily chosen but fixed tracings of the absorption spectrum).

## MATERIALS AND METHODS

Mushroom polyphenol oxidase (tyrosinase; *o*-phenol:$O_2$ oxidoreductase, EC 1.10.3.1) was obtained from Worthington Biochemicals, and dopamine, epinephrine, norepinephrine bitartrate, isoproterenol, *N*-acetyldopamine, and 6-hydroxydopamine, from Sigma Chemical Company. Sodium metaperiodate and components of buffers were reagent grade.

Spectra were recorded with a Beckman Acta III spectrophotometer,[2] which scans at speeds up to 2 nm/sec from higher to lower wavelengths and will record both the ultraviolet and visible spectra in a single tracing. An Aminco DW-2 spectrophotometer[3] was utilized in following more rapid reactions; this instrument scans at rates up to 20 nm/sec from lower to higher wavelengths. Reference cuvettes for tyrosinase oxidation contained all components except substrate. Water was used in reference cuvettes during autoxidation or periodate oxidation. When reactions were run at low temperatures, the cell compartment was continuously flushed with $N_2$ to prevent condensation.

## RESULTS

Dopamine, **3**, norepinephrine, **4**, epinephrine, **5**, and isoproterenol, all analogs of dopa, were oxidized similarly by mushroom tyrosinase to orange chromophores with $\lambda_{max}$ similar to that given for dopachrome **8**, at 302 and 473 nm (3). Thus the dopamine product with $\lambda_{max}$ at 303 and 479 nm can be assumed to be aminochrome, **14**; the product of norepinephrine ($\lambda_{max}$ = 297 and 477 nm), noradrenochrome, **20**; and the epinephrine product ($\lambda_{max}$ = 302 and 480 nm), adrenochrome, **23** (Table 1). The corresponding product from isoproterenol had $\lambda_{max}$ at 305 and 394 nm. Serial 20-nm/sec tracings of the visible spectrum during the tyrosinase-mediated oxidation of dopamine are given in Fig. 3. The 479 nm peak develops quickly, with no evidence for additional chromophores. When this family of curves was subjected to the matrix analysis of Coleman *et al.* (28), a best fit was obtained with the empirical test for two absorbing species in solution (Fig. 4). Thus there was no evidence for the proposed intermediates **12** and **13** in this pathway. Such was the case with the other catecholamines and, as previously reported, with dopa (3).

The *o*-quinone products of dopamine, norepinephrine, and epinephrine were observed, however, when the specific chemical oxidant $NaIO_4$ was employed. In each instance there was the immediate appearance of a yellow chromophore with $\lambda_{max}$ between 380 and 395 nm, which was progressively replaced by the orange chromophore observed during tyrosinase-mediated oxidation of these catechols (Table 1). The visible spectrum obtained during $NaIO_4$ oxidation of dopamine is presented in Fig. 5. In this instance the *o*-quinone species, **12**, is apparent in the peak at 394 nm. The subsequent generation of the 479 nm aminochrome peak is not accompanied by a true isosbestic point, but rather by multiple crossings of succeeding tracings over a span of 10 nm. When these data were subjected to the matrix analysis, a best fit was obtained for three species in solution (Fig. 6).

Similarly, norepinephrine was oxidized to its *o*-quinone, **18**, by $NaIO_4$, in this instance with $\lambda_{max}$ at 384 nm. To observe the transformation of this species to noradrenochrome, it was necessary to reduce the temperature. Matrix analysis, in this instance using data from the ultraviolet as well as the visible spectrum, gave a best fit for three species in solution. The periodate oxidation of epinephrine occurred even more rapidly, with the *o*-quinone, **21**, seen only

[2] The generosity of Dr. A. C. Crumbliss in supplying this instrument is gratefully acknowledged.

[3] This instrument was provided by Dr. I. Fridovich and his colleagues, Dr. R. H. Cassell and Dr. R. E. Lynch, whose gracious cooperation is greatly appreciated.

TABLE 1

*Spectral characteristics of polyphenol oxidation products*

The wavelengths of maximal absorption for the polyphenols and their oxidation products are given for autoxidation at pH 7.4 (0.05 M sodium phosphate), 9.0, 9.5, or 10.5 (0.025 M sodium carbonate), after oxidation by unbuffered sodium periodate (1 Eq for 6-hydroxydopamine; 2 Eq for dopamine, norepinephrine, and epinephrine), or after oxidation by mushroom tyrosinase in 0.05 M sodium phosphate buffer, pH 6.8. Values in parentheses indicate a shift in the $\lambda_{max}$ during continued autoxidation at pH 9.5.

| | $\lambda_{max}$ of unoxidized polyphenol | Oxidizing conditions | $\lambda_{max}$ of product(s) | |
|---|---|---|---|---|
| | | | Ultraviolet | Visible |
| | *nm* | | *nm* | *nm* |
| 6-Hydroxydopamine | 294 | pH 7.4 | 272 | 492 |
| | | pH 9.0 | 270 | |
| | | pH 9.5 | 272 | |
| | | $NaIO_4$ | 270 | 491 |
| | | Tyrosinase, pH 6.8 | 270 | 488 |
| Dopamine | 283 | pH 9.5 | 300 | 450 (431) |
| | | pH 10.5 | 303 | |
| | | $NaIO_4$ | 303 | 394, 479 |
| | | Tyrosinase, pH 6.8 | 303 | 479 |
| Norepinephrine | 283 | pH 9.5 | | 453 (456) |
| | | pH 10.5 | 297 | |
| | | $NaIO_4$ | 296 | 384, 482 |
| | | Tyrosinase, pH 6.8 | 297 | 477 |
| Epinephrine | 283 | pH 9.5 | | 486 |
| | | pH 10.5 | 303 | |
| | | $NaIO_4$ | 302 | 387, 483 |
| | | Tyrosinase, pH 6.8 | 302 | 480 |

in the first tracing, again at reduced temperatures. Matrix analysis again showed three absorbing species in solution.

Recordings of the ultraviolet spectrum shed light on the identity of the third species observed in addition to the *o*-quinone (**12**, **18**, **21**) and the cyclized quinone (**14**, **20**, **23**). The addition of 1 Eq of $NaIO_4$ resulted in the appearance of the ultraviolet peak for the cyclized quinone but also in the persistence of the catechol, as had been observed with the periodate oxidation of dopa (3). Therefore the third species detected by the matrix analysis was the catechol **3**, **4**, or **5** itself rather than leukoaminochrome, **13**, leukonoradrenochrome, **19**, or leukoadrenochrome, **23**. The latter species must be readily oxidized and very transient entities in solution.

The spectra obtained during the autoxidation of dopamine, norepinephrine, and epinephrine at high pH mimicked those obtained during oxidation mediated by mushroom tyrosinase. In each instance matrix analysis of the ultraviolet or visible spectrum gave a best fit for two species in solution, without evidence for the *o*-quinone or leukochrome intermediates. As the periodate oxidation of each gave evidence for persistence of the catechol, so did the catechol persist during autoxidation at high pH. Notable differences in the rates of oxidation were observed among the three catecholamines. The autoxidation of dopamine was much more rapid than that of norepinephrine and epinephrine (Table 2). On the other hand, the genesis of the ultraviolet peak ascribed to the cyclized quinone during periodate oxidation occurred in the reverse order, with more rapid appearance of this species with epinephrine (Table 2). From the first observation, dopamine is obviously the most readily autoxidized. From the second, it is apparent that while oxidation of the catechols **3**, **4**, and **5** to the *o*-quinones **12**, **18**, and **21** with periodate

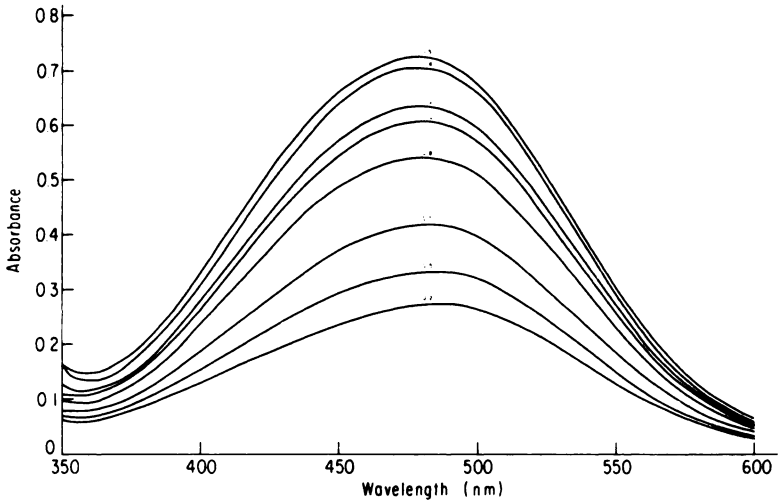


FIG. 3. *Tyrosinase-mediated oxidation of dopamine*

Dopamine at a final concentration of 0.5 mM was added to tyrosinase, 0.25 mg/ml in 0.05 M sodium phosphate, pH 6.8, at 22°. Spectra were recorded at 20 nm/sec, the tracings beginning at the times indicated on each curve in minutes.

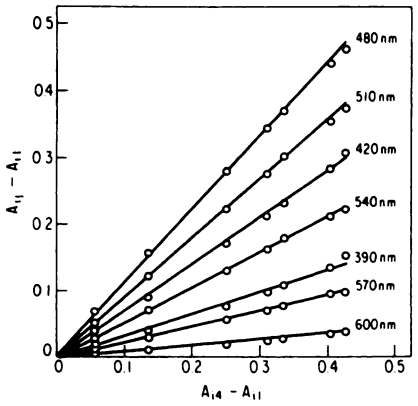


FIG. 4. *Matrix analysis of tyrosinase-mediated oxidation of dopamine*

The empirical test for two species given by Coleman *et al.* (28) and illustrated previously (3) is shown for the oxidation of dopamine in the presence of mushroom tyrosinase. In this analysis $A_{ij}$ is the absorbance at wavelength $i$ obtained during tracing $j$, e.g., $A_{34}$ is the absorbance at 480 nm obtained during the fourth tracing ($t$ = 0.8 min). In the test for two species, ($A_{ij} - A_{i1}$) was calculated at eight wavelengths for the eight tracings illustrated in Fig. 3 and plotted against values for $i$ = 4 (490 nm). The test for two species requires that a family of straight lines pass through the origin as shown.

probably occurs at roughly the same rate, the rates of cyclization to **13**, **19**, and **22** must vary, being greatest for epinephrine and least for dopamine.

One interesting feature was observed in the visible spectra during autoxidation at high pH. The $\lambda_{max}$ values of aminochrome, **14**, and noradrenochrome, **20**, were approximately 30 nm less than observed during tyrosinase- or periodate-mediated oxidation, while that of adrenochrome, **23**, was the same (Table 1). The explanation for this is seen in Fig. 2. The substituent on the imino nitrogen $R_3$ in aminochrome, **14**, and noradrenochrome, **20**, is a proton that will dissociate at high pH, while $R_3$ in adrenochrome is a methyl group. Thus the $\lambda_{max}$ observed for **14** and **20** at high pH is the anion of **28** (Fig. 2), while that at neutral pH (Table 1) is the dipolar ion represented by **28**. Obviously, adrenochrome remains a dipolar ion at high pH.

Sequential recordings of the visible spectrum during the autoxidation of 6-hydroxydopamine at pH 7.4 yielded only a single peak at 492 nm. The oxidation of 6-hydroxydopamine by $NaIO_4$ occurred too rapidly to be studied at the concentrations required for visualization in the visible spectrum, but the several tracings obtained before completion of the reactions again showed the appearance of the 492 nm peak without evidence of other maxima.

Serial tracings of the ultraviolet spec-

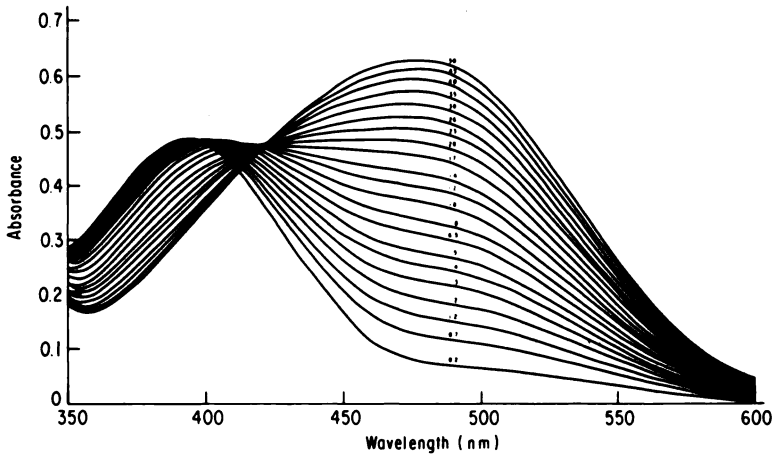


FIG. 5. *Oxidation of dopamine by sodium periodate*

In this experiment 0.5 mM dopamine was oxidized with 0.5 mM $NaIO_4$, 1 Eq instead of 2, to illustrate better the 394 nm peak. The reaction was followed at 22° with tracings at 20 nm/sec, beginning at the times indicated in minutes. The reaction of dopamine with 2 Eq of $NaIO_4$ was similar, except that the 394 nm peak was replaced by the 478 nm peak more rapidly.

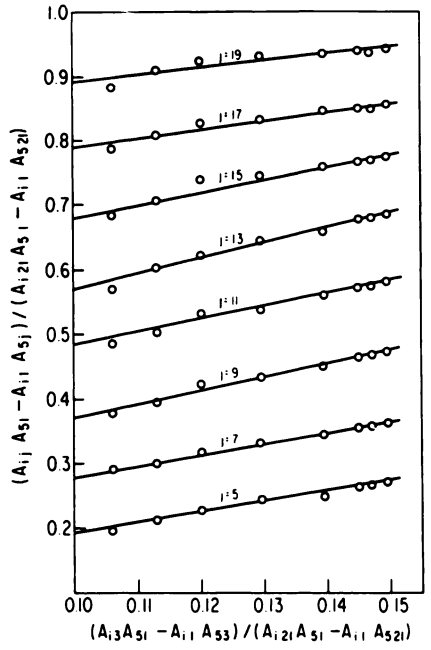


FIG. 6. *Matrix analysis of sodium periodate oxidation of dopamine*

The quantity $(A_{ij}A_{51} - A_{i1}A_{5j})/A_{i21}A_{51} - A_{i1}A_{521})$ was calculated for the odd-numbered tracings illustrated in Fig. 5, with $i$ = 370, 390, 410, 430, 450, 470, 490, 510, 530, and 550 nm. Values for $j$ = 5–19 were plotted against values for $j$ = 3, yielding this family of straight lines, as given by Coleman *et al.* (28). In the test for two species straight lines were not obtained. A similar group of straight lines was obtained when the curves for dopamine plus 2 Eq were subjected to the test for three species in solution, both when the ultraviolet and when the visible spectra were analyzed.

trum, however, revealed notable differences between autoxidation and periodate oxidation of 6-hydroxydopamine. During autoxidation at pH 7.4, a progressive shift of the maximum of unoxidized 6-hydroxydopamine from 294 nm to 272 nm occurred over 16 min at 5°. This was accompanied by sharp isosbestic points at 293 and 316 nm (Fig. 7). Periodate oxidation of 6-hydroxydopamine occurred much more rapidly, going to completion in 2 min at 5° (Fig. 8). Serial tracings again revealed a shift of the $\lambda_{max}$ from 294 and 272 nm. In addition to the isosbestic points at 291 and 316 nm there was an isosbestic point at 242 nm. Matrix analysis of the two processes disclosed that in autoxidation 6-hydroxydopamine, **15**, is oxidized to the 272 and 492 nm chromophore **17**, without evidence for intermediate species (Fig. 9), whereas periodate oxidation of 6-hydroxydopamine gave a best fit with the test for three species in solution, viz. **15**, **16**, and **17** (Fig. 10).

The *p*-quinone product, of 6-hydroxydopamine **17**, was found to shift its ultraviolet $\lambda_{max}$ to higher wavelengths over several hours at 24°, but this process was attended by the increase in general absorbance that

accompanies polymerization. The appearance of a shoulder at 300–305 nm suggested conversion of the *p*-quinone, **17**, to aminochrome, **14**. A technique previously employed to demonstrate cyclization of the *p*-quinone product of topa, **11**, to dopachrome, **8** (3), was used in order to document that cyclization of **17** to **14** also occurred. The *p*-quinone, **17**, produced either by autoxidation or via tyrosinase-mediated oxidation, was loaded on a Sephadex G-25 column and eluted with water until the red band was near the end of the column. Then flow was stopped, and the column was left for 3 days at 4° in the dark. At the end of this period the color of the band had changed from red to orange, and elution of the band yielded aminochrome, **14**, with $\lambda_{max}$ at 303 and 479 nm. The binding of the *p*-quinone, **17**, to Sephadex apparently allowed ring enclosure to occur while impairing polymerization.

TABLE 2

*Autoxidation vs. periodate oxidation of catecholamines*

The rate of development of the ultraviolet maximum (303 nm for dopamine and epinephrine; 297 nm for norepinephrine) was determined at 0.067 mM in 0.025 M sodium carbonate buffer, pH 10.5, and during oxidation by 2 Eq of sodium periodate at 37°.

| Catecholamine | $\Delta A_{max}$ | |
|---|---|---|
| | pH 10.5 | $NaIO_4$ |
| | $min^{-1}$ | |
| Dopamine | 0.175 | 0.145 |
| Norepinephrine | 0.029 | 0.157 |
| Epinephrine | 0.022 | 0.197 |

## DISCUSSION

These studies provide spectrophotometric evidence for the pathways by which dopamine, **3**, norepinephrine, **4**, and epinephrine, **5**, can be oxidized to aminochrome, **14**, noradrenochrome, **20**, and adrenochrome, **23**, through exposure to oxygen at basic pH. This process of autoxidation could thus be postulated to occur under physiological conditions within the cell, although at slower rates. Additionally, oxidative processes that result in the production of superoxide radicals, e.g., xanthine oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, and NADPH–cytochrome *c* reductase, also promote the oxidation of the catecholamines to quinone products (29). Thus an as yet undefined proportion of catecholamines may be oxidized by the routes given in Fig. 1, thereby

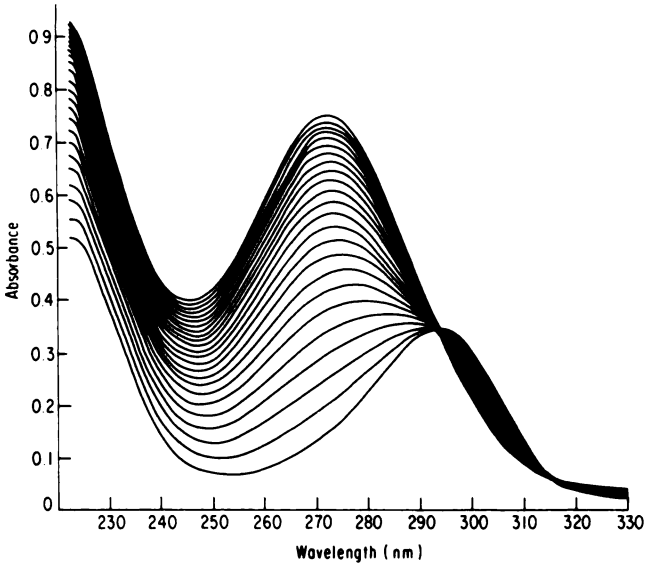


FIG. 7. *Autoxidation of 6-hydroxydopamine*

6-Hydroxydopamine in water was added to 0.045 M sodium phosphate, pH 7.4, to a final concentration of 0.1 mM. Each of the tracings of the ultraviolet spectrum obtained at 5° is illustrated, the initial tracing being that of unoxidized 6-hydroxydopamine with $\lambda_{max}$ at 294 nm.

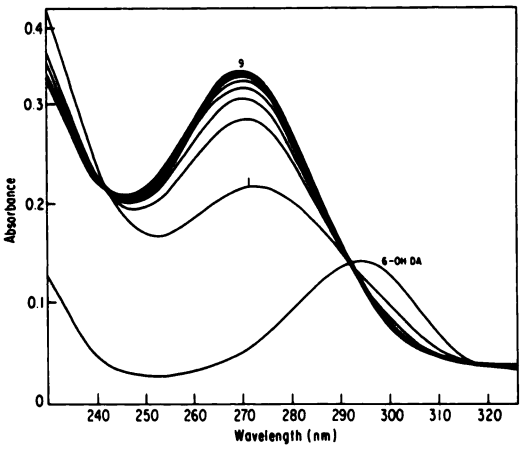


FIG. 8. *Oxidation of 6-hydroxydopamine by sodium periodate*

6-Hydroxydopamine was dissolved in water at 0.033 mM, and the spectrum was recorded ($\lambda_{max}$ = 294 nm). Then 1 Eq of $NaIO_4$ was added with rapid mixing, and sequential 20-nm/sec tracings of the ultraviolet spectrum were recorded at 16-sec intervals at 5°. The first and ninth tracings after the addition of $NaIO_4$ are labeled.

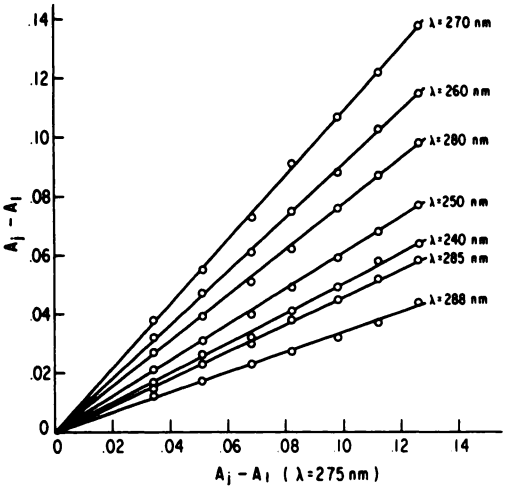


FIG. 9. *Matrix analysis of autoxidation of 6-hydroxydopamine*

The data illustrated in Fig. 7 were analyzed by the test for two absorbing species in solution as described in the legend to Fig. 4. Again, the straight lines obtained pass through the origin. When the data in Fig. 7 were analyzed for three species, straight lines were not obtained.

providing the quinone species that polymerize to form neuromelanin. Neuromelanin could then be viewed as a waste product of catecholamine metabolism, accumu-

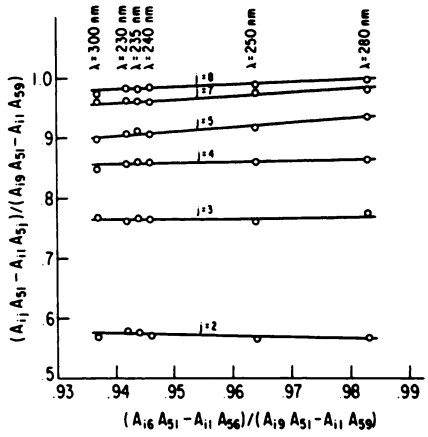


FIG. 10. *Matrix analysis of periodate oxidation of 6-hydroxydopamine*

The test for three absorbing species in solution is shown for the data given in Fig. 8, as described in the legend to Fig. 6. If a family of straight lines is obtained, as is the case here, a minimum of three absorbing species in solution is indicated. Test for two species using the data in Fig. 8 produced a set of straight lines that did not pass through the origin.

lating progressively within the cytoplasm of the neuron with the passage of time. Numerous factors would influence the quantity of catecholamine carbon atoms contributed to the neuromelanin polymer: viz. the presence of reducing substances, such as ascorbate, or the availability of reactive nucleophiles, such as sulfhydryl groups of glutathione or proteins (8, 9). Certainly the relative contribution of these oxidative pathways to catecholamine catabolism must be small compared with *O*-methylation and deamination, since up to 90% of an intravenously administered dose of epinephrine can be recovered in the urine either unmetabolized or as products resulting from *O*-methylation and/or deamination (30, 31). The author's experience as a neuropathologist confirms the sluggish nature of neuromelanin deposition; until a patient is at least 6 years old there is insufficient pigment in the substantia nigra to be detected without the aid of a microscope.

In the oxidation of the catecholamines **3**, **4**, and **5** the expected *o*-quinone intermediates **12**, **18**, and **21** were not evident. Nor did examination of absorption spectra or matrix analysis support the intervention of the leukochromes **13**, **19**, and **22** in autox-

idation. Oxidation of the catecholamines by $NaIO_4$, on the other hand, resulted in the production of transient yellow chromophores with $\lambda_{max}$ values between 380 and 395 nm. Their identification is supported by the $\lambda_{max}$ of 4-(2-*N*-acetylaminoethyl)-1,2-benzoquinone and other 1,2-benzoquinones in aqueous solutions (3). The *o*-quinones generated by $NaIO_4$ oxidation were seen to undergo rapid cyclization in the order **21** > **18** > **12**, resulting in the aminochromes **23**, **20**, and **14**. That the *o*-quinones **6** (3), **12**, **18**, and **21** can be observed spectrophotometrically during periodate oxidation but not during autoxidation indicates that the former process occurs more rapidly and that in autoxidation the first step, the oxidation of catecholamines **3**, **4**, and **5** to the *o*-quinones **12**, **18**, and **21**, is rate-limiting. The existence of *o*-quinone **21** was previously established during the electrochemical oxidation of epinephrine, **5**, by Hawley *et al.* (24), and that of **12**, in a similar study by Tse *et al.* (9). The use of periodate in these studies has allowed the first demonstration of the *o*-quinones **12**, **18**, and **21** and the first demonstration of their subsequent cyclization and oxidation to **14**, **20**, and **23** by absorption spectroscopy.

The differences in rates of cyclization of the *o*-quinones **12**, **18**, and **21** observed in these and other studies (24) imply that especially the dopamine *o*-quinone, **12**, may have the opportunity to react with external nucleophiles rather than undergo internal cyclization (9). That this is a potentially important factor in the cytotoxicity of catecholamines is discussed in the following paper (4).

The present studies also support the existence of the pathway **15**, **17**, **14**. That the red chromophore with $\lambda_{max}$ at 272 and 492 nm, resulting from the autoxidation of 6-hydroxydopamine or its periodate- or tyrosinase-mediated oxidations, is the *p*-quinone **17** is supported by the work of Swan, who reported 270 and 495 nm as the $\lambda_{max}$ of this species. The subsequent cyclization of **17** to aminochrome **14** was also observed by Swan (16).

A chromophore with the spectral properties of *o*-quinone **16** was not observed. In previous studies the corresponding topa *o*-quinone, **10**, was seen as a transient species after periodate oxidation of topa with $\lambda_{max}$ at 265 and 465 nm. *o*-Quinone **10** rapidly tautomerized to the more stable *p*-quinone, **11**, with $\lambda_{max}$ at 271 and 485 nm (3). The only evidence in the experiments shown here for *o*-quinone **16**, which would be the predicted initial product of periodate oxidation of 6-hydroxydopamine (3, 27), was the appearance of an additional isosbestic point when 6-hydroxydopamine was oxidized with periodate and the observation that the matrix analysis of Coleman *et al.* (28) gave evidence for three rather than two absorbing species in solution.

Thus the conversion of *o*-quinone **16** to *p*-quinone **17** must occur by a very rapid tautomeric rearrangement. This observation suggests that previous experiments regarding the reactivity and cytotoxicity of *o*-quinone **16** (20) were instead observations concerning the *p*-quinone, **17**, as noted above.

This study supports the existence of oxidative pathways for the catecholamines other than *O*-methylation and deamination. These pathways would in concept account for the genesis of polymerizing species in the deposition of the apparently inert macromolecule neuromelanin in catecholamine neurons. But in addition, as discussed in the following paper, the oxidative pathways provide the cell with potentially toxic products, with the quinones themselves variably reactive with nucleophiles within the cell and the process of autoxidation resulting in the genesis of free radical species (4).

These experiments also demonstrate that 6-hydroxydopamine can potentially contribute its carbon atoms to neuromelanin via oxidation and cyclization to aminochrome. Thus these data are in agreement with previous observations by Harley-Mason, Swan, and others (14–18).

## REFERENCES

1. Barden, H. (1969) *J. Neuropathol. Exp. Neurol.* **28**, 419–441.
2. Rodgers, A. D. & Curzon, G. (1975) *J. Neurochem.* **24**, 1123–1129.
3. Graham, D. G. & Jeffs, P. W. (1977) *J. Biol. Chem.* **252**, 5729–5734.

4. Graham, D. G., Tiffany, S. M., Bell, W. R., Jr. & Gutknecht, W. F. (1978) *Mol. Pharmacol.*, **14**, 644–653.
5. Jonsson, G., Malmfors, T. & Sachs, C., eds. (1975) *Chemical Tools in Catecholamine Research*, American Elsevier, New York.
6. Senoh, S., Creveling, C. R., Udenfriend, S. & Witkop, B. (1959) *J. Am. Chem. Soc.*, **81**, 6236–6240.
7. Raper, H. S. (1928) *Physiol. Rev.*, **8**, 245–282.
8. Scheulen, M., Wollenberg, P., Bolt, H. M., Kappas, H. & Remmer, H. (1975) *Biochem. Biophys. Res. Commun.*, **66**, 1396–1400.
9. Tse, D. C. S., McCreery, R. L. & Adams, R. N. (1976) *J. Med. Chem.*, **19**, 37–40.
10. Sachs, C. & Jonsson, G. (1975) *Biochem. Pharmacol.*, **24**, 1–8.
11. Kostrzewa, R. M. & Garey, R. E. (1976) *J. Pharmacol. Exp. Ther.*, **197**, 105–118.
12. Senoh, S. & Witkop, B. (1959) *J. Am. Chem. Soc.*, **81**, 6231–6235.
13. Saner, A. & Thoenen, H. (1971) *Mol. Pharmacol.*, **7**, 147–154.
14. Harley-Mason, J. (1953) *J. Chem. Soc.*, 200–203.
15. Borchardt, R. T. (1975) *Mol. Pharmacol.*, **11**, 436–449.
16. Swan, G. A. (1976) *J. Chem. Soc. Perkin Trans. I*, 339–341.
17. Liang, Y. O., Plotsky, P. M. & Adams, R. N. (1977) *J. Med. Chem.*, **20**, 581–583.
18. Blank, C. L., Kissinger, P. T. & Adams, R. N. (1972) *Eur. J. Pharmacol.*, **19**, 391–394.
19. Borchardt, R. T., Smissman, E. E., Nerland, D. & Reid, J. R. (1976) *J. Med. Chem.*, **19**, 30–37.
20. Heikkila, R. E., Mytilineou, C., Cote, L. & Cohen, G. (1973) *J. Neurochem.*, **20**, 1345–1350.
21. Wehrli, P. A., Pigott, F., Fischer, U. & Kaiser, A. (1972) *Helv. Chim. Acta*, **55**, 3057–3061.
22. Hochstein, P. & Cohen, G. (1960) *J. Neurochem.*, **5**, 370–378.
23. Maguire, M. E., Goldmann, P. H. & Gilman, A. G. (1974) *Mol. Pharmacol.*, **10**, 563–581.
24. Hawley, M. D., Tatawawadi, S. V., Piekarski, S. & Adams, R. N. (1967) *J. Am. Chem. Soc.*, **89**, 447–450.
25. Harrison, W. H., Whisler, W. W. & Hill, B. J. (1968) *Biochemistry*, **7**, 3089–3094.
26. Bu'Lock, J. D. & Harley-Mason, J. (1951) *J. Chem. Soc.*, 712–716.
27. Wiedman, S. W. & Kaiser, E. T. (1966) *J. Am. Chem. Soc.*, **88**, 5820–5827.
28. Coleman, J. S., Varga, L. P. & Mastin, S. H. (1970) *Inorg. Chem.*, **9**, 1015–1020.
29. Fridovich, I. (1975) *Annu. Rev. Biochem.*, **44**, 147–159.
30. Axelrod, J. (1960) *Ciba Found. Symp. Adrenergic Mechanisms*, 28–39.
31. Kirshner, N. (1960) *Ciba Found. Symp. Adrenergic Mechanisms*, 45–56.