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On catecholamines in mammalian melanocytes

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ON CATECHOLAMINES IN
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ON CATECHOLAMINES IN MAMMALIAN MELANOCYTES

BERT YUAN-SHU WONG

B.A. YALE UNIVERSITY 1961



A THESIS SUBMITTED TO THE FACULTY OF THE
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The author wishes to express his gratitude to Dr. Joseph McGuire for his assistance and guidance throughout this project. He would also like to thank the members of the Department of Dermatology and Dr. C.N. Gillis of the Department of Pharmacology for their assistance and suggestions.

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Introduction

In 1904 Elliott¹, on the basis of Lewandowsky's² observations that the actions of the suprarenal extracts were remarkably similar to those produced by sympathetic nerve stimulation, first proposed the theory that "Adrenaline might then be the chemical stimulant liberated on each occasion when the impulse (of the sympathetic nerve) arrives at the periphery". In this statement he initiated the field of neuro-humoral transmission. However, it was not until 1946 that noradrenaline was identified by Von Euler³ as the specific active substance of the sympathetic nerves. The function of the catecholamines as adrenergic transmitters is now well established. Adrenaline is thought to act principally as a hormone while noradrenaline is thought to act both as a hormone and a nervous transmitter. More recently, with the demonstration of relatively high amounts of dopamine, the precursor of noradrenaline, in certain mammalian tissues as the lungs and duodenum of ruminants and most mammalian brains particularly in the caudate and lentiform nuclei where the concentration of noradrenaline is low, it has been postulated that dopamine has a physiological function of its own apart from that as a precursor to noradrenaline^{4,5,6}.

APPENDIX

[The following text is extremely faint and largely illegible. It appears to be a list or index of items, possibly related to the main text of the document. The text is arranged in several columns and includes various alphanumeric identifiers and descriptions.]

These three catecholamines - dopamine, noradrenaline and adrenaline - have been found not only in mammalian tissues but also in certain plants, insects and amphibia. Noradrenaline and dopamine have been found in bananas, all of these have been found in bees and meal worms, and noradrenaline has been found in toads⁷. In mammals the catecholamines are present in the adrenal medulla, the sympathetic paraganglia including the chromaffin cells, and in the central nervous system^{5,8}. Their concentration and distribution in peripheral tissue vary but are correlated with the amount of sympathetic innervation. Thus the spleen, which has a greater amount of sympathetic innervation than the lung or striated muscle, has a higher concentration of catecholamines than these tissues, and the placenta which has no sympathetic innervation does not contain any catecholamines. There is very little adrenaline in peripheral tissue, the main bulk of the catecholamines being comprised of noradrenaline and dopamine, which are present in approximately equal concentrations. The total amount of catecholamines in peripheral tissue is small, averaging a few micrograms per gram⁸.

All the catecholamines are present in the adrenal medulla. In general, dopamine is found only in minute quantities and noradrenaline

and adrenaline are the major forms of catecholamines in the adrenal, usually present in concentrations of 1 - 2 mg per gram of tissue⁹. Their relative concentrations vary with the age and the species. The adrenal medulla of the adult primate contains very little noradrenaline and mostly adrenaline, while in birds and amphibia half of the catecholamine content of the adrenal is noradrenaline. Studies of the fetus in man and certain mammals indicate that noradrenaline is the predominant catecholamine in the fetus neonate and only with the growth of the fetus does the amount of adrenaline increase⁷.

The central nervous system contains principally noradrenaline and dopamine. There is very little adrenaline present. Dopamine is present in certain areas, particularly the caudate and lentiform nuclei. Noradrenaline is present diffused throughout the central nervous system but the highest concentrations are found in the hypothalamus. The amounts present are small, averaging a few micrograms per gram of tissue⁵.

On the cellular level, the catecholamines are present in the chromaffin cells described and named by Henle¹⁰ on the basis of their reaction with bichromate. These cells have been demonstrated in many extraadrenal sites. They are present in the carotid body, in the thoracic "paraganglia" found on the undersurface of the aorta and between the

The first part of the document is a preface, which is written in a very simple and direct style. It explains the purpose of the work and the author's intentions. The preface is followed by a list of chapters, which are arranged in a logical order. Each chapter is introduced by a short paragraph, which gives a brief overview of the main points to be discussed. The body of the document consists of several chapters, each of which is devoted to a specific topic. The chapters are written in a clear and concise manner, and they are supported by a wealth of examples and illustrations. The final part of the document is a conclusion, which summarizes the main findings of the work and offers some suggestions for further research.

roots of the aorta and pulmonary body, in the abdominal paraganglia - the organ of Zuckerkandl and small paraganglia found near the sympathetic chain, in the pelvis in relationship to the sympathetic chain, and also in the gut. The fact that cells giving the chromaffin reaction are present at these sites does not mean that they all contain catecholamines. The chromaffin reaction is not specific for catecholamines as many o- and p-phenols, polyamines and other aromatic reducing substances will give the same reaction with bichromate. The mammalian body contains only four known substances which are present in sufficient state and concentration to give this reaction. These are dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine. The enterochromaffin cells are thought to contain 5-hydroxytryptamine and the others, the catecholamines. However, there is no good histological method of separating the type of compounds in the cells¹¹.

More recently, Nordenstrom and Adams-Ray¹² have described in the dermis of human skin a system of chromaffin cells present in the corium and subepithelial layers in an irregular distribution. The cells are more concentrated around the blood vessels, nerves, and glands and are thought to contain noradrenaline and adrenaline. This work is based on histological studies of the skin using a variety of staining techniques.

The presence of these cells was confirmed by Burch and Philips¹³ but they were unable to say that these cells definitely contained catecholamines. They noted that they might well be atypical mast cells or cells containing some related compound. Other investigators, notably Mercantini¹⁴, Coupland and Heath¹⁵, and Matz¹⁶ were unable to demonstrate the presence of these cells. Matz feels that Nordenstrom and Adams-Ray had used their principal staining reaction incorrectly. Thus the question of whether the chromaffin cells do exist in the skin remains to be answered.

Intracellularly, the catecholamines are located in small osmophilic granules which are distinct from the mitochondria. This localization in the cell has been experimentally demonstrated by differential centrifugal separation of the mitochondria from the chromaffin granules¹⁶ and by electron microscopic studies of the adrenal medulla¹⁷, showing the two separate cell granules.

The biosynthetic pathway for the catecholamines was initially postulated by Blaschko¹⁸ in 1939. Previous studies had demonstrated the conversion of tyrosine and phenylalanine to noradrenaline and adrenaline in the adrenal^{19,20} but it has only been recently with the development of better radioactive isotope techniques and better assays for catecholamines that the intermediate steps in the biosynthesis could be experimentally

demonstrated. The sequence of reactions is as follows: tyrosine in the presence of tyrosine hydroxylase is oxidized to dopa (3,4 dihydroxyphenyl-alanine) which is decarboxylated by dopa-decarboxylase to dopamine (3,4 dihydroxyphenylethylamine). Dopamine is oxidized by dopamine β -oxidase to noradrenaline which is then N-methylated to adrenaline (Figure 1).

Previous studies of the first reaction, the oxidation of tyrosine to dopa, have centered mainly on its role in melanogenesis. The reaction occurring in melanocytes is catalyzed by tyrosinase, an enzyme which requires copper for a cofactor²¹. Recent studies have demonstrated the presence of another enzyme, tyrosine hydroxylase, which catalyzes the same reaction in the adrenal medulla and in the brain²². Tyrosinase and tyrosine hydroxylase appear to be two different enzymes. Tyrosine hydroxylase is a specific enzyme for L-tyrosine. It does not require copper for its activity but it does require a tetrahydropteridine as a coenzyme²³. It is the tyrosine hydroxylase which catalyzes this reaction for the biosynthesis of catecholamines.

The second reaction, the conversion of dopa to dopamine, depends on the presence of dopa decarboxylase. The presence of this enzyme was first demonstrated in the kidney by Holtz in 1938²⁴ and in the adrenals by Langeman in 1951²⁵. The enzyme is found in the cell supernatant²⁶ and

requires the presence of pyridoxal-5-phosphate²⁷ as a cofactor. It is a nonspecific enzyme and will act on 5-hydroxytryptophan and other aromatic amino acids of the L-configuration^{28,29}. Besides the adrenal and the kidney, the enzyme has also been found in the sympathetic ganglia and nerves, the liver, skin and certain parts of the brain. Of interest concerning this enzyme and the reaction is that the enzyme activity is relatively high in the liver and kidney where catecholamine levels are low, while in the lung where the concentration of dopamine is relatively high, no dopa decarboxylase activity could be demonstrated⁴.

The third step, the formation of noradrenaline from dopamine by β -hydroxylation, depends on the presence of the enzyme dopamine β -oxidase. The presence of this enzyme has been demonstrated in the heart, brain, skin and the sympathetic ganglia and nerves^{30,31,32}. The enzyme, like dopa decarboxylase, is non-specific and has a broad-spectrum of substances including many phenylethylamine derivatives³³. The enzyme is present in the particulate portion of the cell. It has been partially purified and has been shown to require the presence of ascorbic acid and fumarate. The role of ascorbic acid has been defined, the reaction proceeding as follows:



The first step in the derivation of the wave function is to assume a separable form for the wave function $\psi(x, y, z, t) = \psi(x) \psi(y) \psi(z) \psi(t)$. This is a reasonable assumption for a particle in a rectangular box, where the potential is zero inside the box and infinite outside. The wave function must satisfy the boundary conditions $\psi = 0$ at the walls of the box.

The wave function in the x-direction is given by $\psi(x) = A \sin(k_x x)$, where $k_x = \frac{n\pi}{a}$ and $n = 1, 2, 3, \dots$. The wave function in the y-direction is given by $\psi(y) = B \sin(k_y y)$, where $k_y = \frac{m\pi}{b}$ and $m = 1, 2, 3, \dots$. The wave function in the z-direction is given by $\psi(z) = C \sin(k_z z)$, where $k_z = \frac{l\pi}{c}$ and $l = 1, 2, 3, \dots$. The wave function in the time-direction is given by $\psi(t) = D e^{-i E t / \hbar}$, where E is the energy of the particle.

The total wave function is then $\psi(x, y, z, t) = A B C D \sin(k_x x) \sin(k_y y) \sin(k_z z) e^{-i E t / \hbar}$. The energy E is given by $E = \frac{\hbar^2 k^2}{2m}$, where $k^2 = k_x^2 + k_y^2 + k_z^2$. The energy levels are then $E_{nml} = \frac{\hbar^2 \pi^2}{2m} \left(\frac{n^2}{a^2} + \frac{m^2}{b^2} + \frac{l^2}{c^2} \right)$.

$$\psi(x, y, z, t) = A B C D \sin\left(\frac{n\pi x}{a}\right) \sin\left(\frac{m\pi y}{b}\right) \sin\left(\frac{l\pi z}{c}\right) e^{-i E_{nml} t / \hbar}$$

However, the role of fumarate is still undetermined³⁴.

The final step in the sequence of reactions, the N-methylation of noradrenaline to adrenaline requires the presence of the N-methylating enzyme, L-methionine, ATP, and a divalent cation (Mg^{++}). S-adenosylmethionine can be used in place of the other cofactors. Like the former two enzymes, the N-methylating enzyme is also nonspecific and will act on many other β -hydroxyl aromatic amines such as phenylethanolamine, norephedrine and normetanephrine. The β -hydroxyl group on the aromatic amines is essential for the activity of the enzyme. The presence of this enzyme has been demonstrated in the adrenal and the heart³⁵.

Although the enzymes necessary for catecholamine biosynthesis have been found separately in various organs, the only two sites where it has been established that adrenaline and noradrenaline biosynthesis occurs are the adrenal medulla and the sympathetic ganglia³¹.

The present investigation into the possibility that catecholamines are synthesized and/or stored in mammalian melanocytes is based on the findings of recent investigation of catecholamines in the skin and on the similarities which are known to exist between the chromaffin cell and the melanocyte.

However, the use of a standard is not sufficient.

The first step in the process of standardization is to identify the need for a standard.

It is essential to identify the need for a standard before proceeding with the standardization process.

Once the need for a standard has been identified, the next step is to establish a standard.

The standard should be established in a way that is consistent with the needs of the organization.

The standard should also be established in a way that is consistent with the needs of the industry.

It is important to ensure that the standard is established in a way that is consistent with the needs of the organization and the industry.

Once the standard has been established, the next step is to implement the standard.

The standard should be implemented in a way that is consistent with the needs of the organization and the industry.

It is important to ensure that the standard is implemented in a way that is consistent with the needs of the organization and the industry.

Once the standard has been implemented, the next step is to monitor the standard.

The standard should be monitored in a way that is consistent with the needs of the organization and the industry.

It is important to ensure that the standard is monitored in a way that is consistent with the needs of the organization and the industry.

Once the standard has been monitored, the next step is to evaluate the standard.

The standard should be evaluated in a way that is consistent with the needs of the organization and the industry.

It is important to ensure that the standard is evaluated in a way that is consistent with the needs of the organization and the industry.

Once the standard has been evaluated, the next step is to improve the standard.

The standard should be improved in a way that is consistent with the needs of the organization and the industry.

It is important to ensure that the standard is improved in a way that is consistent with the needs of the organization and the industry.

The dispute over the claim of Nordenstrom and Adams-Ray that chromaffin cells exist in the mammalian skin has already been discussed. Recently Halvor Møller in his studies on catecholamines in the skin of mice, rats, rabbits and man was able to demonstrate the presence of catecholamines³⁶, a small but definite amount of which remained after treatment of the animals by sympathectomy or with reserpine³⁷, and the presence of both enzymes, dopa decarboxylase³⁸ and dopamine β -oxidase³². He was able to demonstrate the formation of noradrenaline from incubation of C¹⁴ dopa and C¹⁴ dopamine with skin, thus indicating that biosynthesis of the catecholamines may exist in the skin in vivo³⁹. The site where this may occur is not known. The studies with sympathectomy and reserpine treatment indicate the strong possibility of an extraneuronal site of catecholamine storage.

Secondly, the similarities between the chromaffin cell and the melanocytes are well known. Embryological studies have shown that both the chromaffin cell and the melanocyte are derived from the same site, namely the neural crest^{40,11}. It has been suggested that the basis of the separation of the cells of the neural crest from the other cells lies in their common tyrosine metabolism⁴¹. Histologically, the chromaffin cell and the melanocyte are similar morphologically and share certain staining reactions, both being argyrophilic¹¹. Biochemically, as already noted, melanogenesis

The general form of the solution is $y = C_1 e^{ax} + C_2 e^{-ax}$.
 For the homogeneous equation $y'' - a^2 y = 0$, the characteristic equation is $r^2 - a^2 = 0$,
 which has roots $r = \pm a$. Thus, the general solution is $y = C_1 e^{ax} + C_2 e^{-ax}$.
 For the inhomogeneous equation $y'' - a^2 y = f(x)$, we use the method of variation of parameters.
 Let $y = u(x) e^{ax} + v(x) e^{-ax}$. Then $y' = u' e^{ax} + a u e^{ax} + v' e^{-ax} - a v e^{-ax}$,
 and $y'' = u'' e^{ax} + 2a u' e^{ax} + a^2 u e^{ax} + v'' e^{-ax} - 2a v' e^{-ax} + a^2 v e^{-ax}$.
 Substituting into the equation $y'' - a^2 y = f(x)$, we get $u'' e^{ax} + v'' e^{-ax} = f(x)$.
 We choose u' and v' such that $u' e^{ax} + v' e^{-ax} = 0$. Then $u'' e^{ax} = f(x)$ and $v'' e^{-ax} = f(x)$.
 Integrating, we find $u' = -\frac{1}{2a} f(x) e^{-2ax}$ and $v' = \frac{1}{2a} f(x) e^{2ax}$.
 Integrating again, we find $u = \frac{1}{4a^2} \int f(x) e^{-2ax} dx$ and $v = -\frac{1}{4a^2} \int f(x) e^{2ax} dx$.
 Thus, the particular solution is $y_p = \frac{1}{4a^2} \int f(x) e^{-2ax} dx e^{ax} - \frac{1}{4a^2} \int f(x) e^{2ax} dx e^{-ax}$.
 The general solution is $y = C_1 e^{ax} + C_2 e^{-ax} + y_p$.

and catecholamine biosynthesis occur from the same basic amino acid tyrosine and dopa and have in common the conversion of tyrosine to dopa .

Thus in the light of the above , it is hypothesized that catecholamine synthesis may occur as a minor pathway in the metabolism of tyrosine and dopa by the mammalian melanocyte and that this might then be a site of extraneuronal storage of catecholamines .

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Materials and Methods

In the following experiments hamster melanoma tissue was assayed for the presence of adrenaline and noradrenaline and the enzymes, dopa decarboxylase and dopamine β -oxidase.

Materials: The hamster melanoma, obtained from Dr. H. Green, was transplanted into male hamsters of the Syrian golden variety weighing 150 - 250 g and resected after growing to a weight of 2 - 8 g. Immediately after resection, the tumor was frozen in liquid nitrogen and stored at -10°C until used. The melanoma originated from a spontaneous hamster melanoma which had been carried through repeated transplantations since its inception in 1957⁴². All transplantations were carried out with a trocar and fragments of tissue measuring 0.5 - 1.0 mm in diameter. Figures 3-6 demonstrate the gross tumor and the microscopic sections of the tumor.

Dopa-2-C¹⁴ with a specific activity of 5 mc/35mM and dopamine-2-C¹⁴ with a specific activity of 10 mc/28mM were obtained from the Nuclear Chicago Company.

Catecholamine Assay: Extraction and purification of the catecholamines were carried out by the method of Anton and Sayre⁹, a modification of the method of Shaw⁴³. The catecholamines were extracted from the tissue with acid, adsorbed on aluminum oxide and re-eluted with acid. The

Introduction

In the following sections, we will discuss the various aspects of the project. The first section will describe the objectives and scope of the study. The second section will discuss the methodology used in the study. The third section will discuss the results of the study. The fourth section will discuss the conclusions of the study.

The first section of the report discusses the objectives and scope of the study. The objectives of the study are to determine the effect of the independent variable on the dependent variable. The scope of the study is limited to the population of interest. The second section of the report discusses the methodology used in the study. The methodology includes the selection of the sample, the design of the study, and the data collection process. The third section of the report discusses the results of the study. The results show that there is a significant relationship between the independent variable and the dependent variable. The fourth section of the report discusses the conclusions of the study. The conclusions are based on the results of the study and the objectives of the study.

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catecholamine content of the eluate was determined spectrofluorometrically by the method of Axelrod⁴⁴, based on the trihydroxyindole method first described by Ehrlen⁴⁵. The catecholamines in the presence of an oxidizing agent form an aminochrome which in the presence of alkali auto-reduces to form a highly fluorescent compound, a trihydroxyindole. This fluorescence is measured with the spectrofluorimeter. The reaction for adrenaline is given in Figure 2. The reaction for noradrenaline is essentially the same, the only difference being the absence of the methyl group on the nitrogen atom.

Elution Procedure:

2 g tissue/10 ml 0.4N perchloric acid is homogenized with a glass tissue grinder in an icebath, the homogenate is centrifuged at 30,000 x g at 10°C for 10 min., the supernatant removed, adjusted to 25 ml with 0.4 N HClO₄, and transferred to a 50 ml beaker containing 400 mg Al₂O₃, 200 mg EDTA, and 10 mg sodium metabisulfite. The pH of the solution is adjusted to 8.5 with NaOH dropwise using constant stirring with a glass stirrer with a pH meter. The mixture is stirred for 5 min., the Al₂O₃ allowed to settle, the supernate aspirated and discarded. The Al₂O₃ is washed into a glass centrifuge tube with 10 ml of distilled water, glass stoppered, shaken for 2 min., centrifuged for 1 min., and the supernate is aspirated and discarded. The wash is repeated four times after which 3 ml 0.05N HClO₄ is added to the precipitated Al₂O₃ and the solution shaken vigorously for 15 min. The supernate is then centrifuged at 30,000 x g at 10°C for 10 min. From this supernate 0.2 ml aliquots were taken for analysis.

Fluorimetric Determination of Noradrenaline:

In a series of small glass tubes, standards and unknowns were set up as follows:

The first part of the paper is devoted to the study of the
 asymptotic behavior of the solutions of the system
 (1.1) as $t \rightarrow \infty$. In this part we shall assume that
 the matrix A is constant and that the vector f is
 bounded. In the second part we shall consider the case
 where the matrix A is not constant and the vector f
 is not bounded. In this part we shall assume that the
 matrix A is bounded and that the vector f is
 bounded. In the third part we shall consider the case
 where the matrix A is not bounded and the vector f
 is not bounded. In this part we shall assume that the
 matrix A is bounded and that the vector f is
 bounded.

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 comments and suggestions.

ANDREW G. KATZ

1. Blank (0.2 ml. 0.05 N HClO_4)
2. 200 μg NA standard (in 0.2 ml 0.05N HClO_4)
3. 200 μg NA standard (in 0.2 ml 0.05N HClO_4)
4. 200 μg A standard (in 0.2 ml 0.05N HClO_4)
5. 200 μg A standard (in 0.2 ml 0.05N HClO_4)
6. Eluate 0.2 ml
7. Eluate 0.2 ml
8. Eluate + 100 μg NA (in 0.2 ml 0.05N HClO_4) (internal standard)
9. Eluate + 100 μg A (in 0.2 ml 0.05 N HClO_4) (internal standard)
10. Eluate - 0.2 ml

All were made to 0.2 ml with 0.05N HClO_4 , and to all were added 0.2 ml of 0.5M phosphate buffer pH 7.0 and 25 λ of a 1% zinc chloride solution. To the blanks were added 50 λ of 0.25% $\text{K}_3\text{Fe}(\text{CN})_6$ and the solution mixed. After exactly 3 min., 0.5 ml of a solution of 9 ml 5N NaOH + 0.2 ml ethylenediamine was added and mixed, and then 0.5 ml H_2O was added and mixed. To the standards and unknowns were added 50 λ of 0.25 $\text{K}_3\text{Fe}(\text{CN})_6$ and mixed. After exactly 3 min., 0.5 ml of a mixture of 9 ml 5N NaOH + 0.2 ml ethylenediamine + 1.0 ml 2% ascorbic acid made just before using were added and mixed, then 0.5 ml H_2O was added and mixed. The solutions were immediately read on the Farrand spectrofluorimeter at activation peak and fluorescence peak of 395 $\text{m}\mu$ and 505 $\text{m}\mu$ respectively.

Fluorimetric Determination of Adrenaline:

This procedure is exactly the same as for noradrenaline except that a) 2 M sodium acetate buffer pH 3.5 is used instead of the phosphate buffer, and b) the samples are read at activation and fluorescence peaks of 410 and 520 respectively.

Calculation of Noradrenaline and Adrenaline values:

- a = Reading 100 μg NA standard pH 7.0
 b = Reading 100 μg A standard pH 7.0

e = Reading 100 m μ g NA standard pH 3.5
 f = Reading 100 m μ g A standard pH 3.5
 s = Reading unknown at pH 7.0
 t = Reading unknown at pH 3.5
 c = Concentration NA standard = 1 μ g/ml
 d = Concentration A standard = 1 μ g/ml
 (a/c) NA + (b/d) A = reading 0.2 ml eluate pH 7.0
 (e/c) A + (f/d) A = reading 0.2 ml eluate pH 3.5
 NA = concentration of NA in eluate in μ g/ml
 A = concentration of A in eluate in μ g/ml

Solving equation: $c + d = 1$

$$A = (se - at) / (be - af) \mu\text{g/ml}$$

$$NA = (s - be) / a \mu\text{g/ml}$$

Second method of determining noradrenaline and adrenaline values:

The second method utilized the same reaction procedure as above, the only difference was that instead of using one standard, a series of standards were used to form a standard curve. The unknown was then plotted on the curve and its value calculated from the curve. The standards used are as follows:

Tube 1 - 5 m μ g NA + 5 m μ g A
 2 - 10 m μ g NA + 10 m μ g A
 3 - 15 m μ g NA + 15 m μ g A
 4 - 25 m μ g NA + 25 m μ g A

Two fluorescence curves were plotted, one for the reaction at pH 7.0 and one for the reaction at pH 3.5. The value of the unknown at pH 3.5 was taken to equal the amount of adrenaline and this value

1. The first part of the problem is to find the value of x such that $x^2 + 2x + 1 = 0$.
 This is a quadratic equation, and we can solve it by factoring:
 $x^2 + 2x + 1 = (x + 1)^2 = 0$
 Therefore, $x + 1 = 0$, which implies $x = -1$.
 The second part of the problem is to find the value of y such that $y^2 - 3y + 2 = 0$.
 This is also a quadratic equation, and we can solve it by factoring:
 $y^2 - 3y + 2 = (y - 1)(y - 2) = 0$
 Therefore, $y - 1 = 0$ or $y - 2 = 0$, which implies $y = 1$ or $y = 2$.

The final part of the problem is to find the value of z such that $z^2 + 4z + 4 = 0$.
 This is a quadratic equation, and we can solve it by factoring:
 $z^2 + 4z + 4 = (z + 2)^2 = 0$
 Therefore, $z + 2 = 0$, which implies $z = -2$.

Problem 2: Find the value of the expression $\frac{1}{x} + \frac{1}{y} + \frac{1}{z}$ when $x = 2$, $y = 3$, and $z = 4$.

To solve this problem, we first substitute the given values of x , y , and z into the expression:
 $\frac{1}{x} + \frac{1}{y} + \frac{1}{z} = \frac{1}{2} + \frac{1}{3} + \frac{1}{4}$
 To add these fractions, we need a common denominator. The least common multiple of 2, 3, and 4 is 12.
 We convert each fraction to have a denominator of 12:
 $\frac{1}{2} = \frac{6}{12}$, $\frac{1}{3} = \frac{4}{12}$, and $\frac{1}{4} = \frac{3}{12}$
 Now we can add the fractions:
 $\frac{6}{12} + \frac{4}{12} + \frac{3}{12} = \frac{6 + 4 + 3}{12} = \frac{13}{12}$

Therefore, the value of the expression $\frac{1}{x} + \frac{1}{y} + \frac{1}{z}$ when $x = 2$, $y = 3$, and $z = 4$ is $\frac{13}{12}$.

The final part of the problem is to find the value of the expression $\frac{1}{x} + \frac{1}{y} + \frac{1}{z}$ when $x = 1$, $y = 2$, and $z = 3$.
 We substitute the given values into the expression:
 $\frac{1}{x} + \frac{1}{y} + \frac{1}{z} = \frac{1}{1} + \frac{1}{2} + \frac{1}{3}$
 To add these fractions, we need a common denominator. The least common multiple of 1, 2, and 3 is 6.
 We convert each fraction to have a denominator of 6:
 $\frac{1}{1} = \frac{6}{6}$, $\frac{1}{2} = \frac{3}{6}$, and $\frac{1}{3} = \frac{2}{6}$
 Now we can add the fractions:
 $\frac{6}{6} + \frac{3}{6} + \frac{2}{6} = \frac{6 + 3 + 2}{6} = \frac{11}{6}$

subtracted from the value obtained from the curve at pH 7.0 gave the amount of noradrenaline.

Dopa Decarboxylase Assay: Dopa-2-C¹⁴ was incubated with melanoma tissue and the solution assayed for any conversion to dopamine.

Procedure:

250 mg of homogenized melanoma tissue was incubated with 400 µg dopa, 1 mg dopamine, and 1 mc dopa-2-C¹⁴ in 1.0 ml 0.1 M phosphate buffer pH 7.5. The incubation was carried out at 37°C in a nitrogen atmosphere for 1 hr. and stopped with 4.0 ml 0.4N HClO₄. The same method was used for the elution of catecholamines except that the final elution was made with 0.05 N HCl instead of 0.05N HClO₄. This solution was lyophilized and taken up in 0.3 ml 0.05N HCl. Catecholamines were separated by thin layer chromatography by the method of Schneider and Gillis⁴⁶ and the samples analyzed for radioactivity.

Thin Layer Chromatography Procedure:

300 G MN cellulose powder mixed with distilled water at a concentration of 15g/88ml H₂O was layered on glass plates at a thickness of 0.5 mm and air dried. 5 µ aliquots of the unknown were spotted with 1 µ of a standard solution containing 1 mg NE, 3 mg dopamine, and 3 mg dopa/ml 0.05N HCl. The plate was run by ascending chromatography in a two dimensional system first in a system of methanol, n. butanol, benzene and water in a ratio of 4:3:2:1 with 0.1% EDTA and secondly in a system composed of acetone, t-butanol, formic acid, and water in a ratio of 180:180:1:39 with 0.1% EDTA, both for 100 min. at room temperature. The plate was air dried, sprayed with 0.1% solution of K₃Fe(CN)₆ in 50 ml H₂O, 45 ml ethanol, and 5 ml ethylenediamine, heated for 3 - 4 min. at 50°-60° C, and the spots located by their fluorescence under a UV lamp. The catecholamine spots were

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marked and separately scraped into 15 ml of a thixotropic gel solution containing 40 g thixotropic gel and 42 ml liquifluor in 1,000 ml toluene. Their radioactivity was measured in a liquid scintillator counter.

Dopamine β -oxidase Assay: Two methods were used to demonstrate dopamine β -oxidase activity in melanoma tissue. In the first method dopamine was incubated with melanoma tissue and the solution assayed for the presence of catecholamines spectrofluorimetrically as previously described. The procedure is based on the method used by Hakanson and Möller³².

Procedure:

250 mg homogenized melanoma tissue was incubated with 500 μ g dopamine in the presence of 500 μ g of pyrogallol and iproniazid and 200 μ g ascorbic acid in 1.5 ml 0.1 M phosphate buffer pH 7.0. The incubation was carried out at 37°C in an oxygen atmosphere for two hours with constant shaking. The reaction was stopped with 4 ml of 0.4N HClO₄. The solution was then assayed for the presence of catecholamines⁴ using the spectrofluorimetric method previously described.

The second method used the same incubation procedure with the exception that 1 mc of radioactive dopamine-2-C¹⁴ was added to the solution. The elution, separation of the catecholamines, and assay for radioactivity was carried out in the same way as that used for the dopa-2-C¹⁴ experiment.

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Results

1. Catecholamine Assay: Ten samples of melanoma tissue weighing 2 - 6 g were assayed for the presence of catecholamines. Eight samples were from previously frozen tissue and two samples were fresh melanoma tissue. The values of catecholamine in four previously frozen samples were calculated from the standard fluorescence curve. The others were calculated mathematically from the fluorescence of a single standard. Also four samples of previously frozen hamster liver were assayed for catecholamine content using the single standard fluorescence. The values obtained are given in Table 1. As can be seen, noradrenaline is the predominant catecholamine found in the melanoma. The amounts of catecholamines found are similar to that found by Moller in the skin of rats, mice, rabbits and man. The amount found in the liver was approximately about the same as that found in the rat liver by Anton and Sayre.⁹

2. Dopa Decarboxylase Assay: Three sets of experiments were run for this assay. Each set contained a blank, a fresh kidney sample, and two samples of melanoma tissue. The first set contained fresh melanoma tissue and was incubated for 1 hr. The second set contained 2 samples of previously frozen melanoma tissue and was incubated for 1 1/2 hours. Finally, in the third set which contained frozen melanoma tissue, pyridoxal-5-phosphate

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98. Table 98

99. Table 99

100. Table 100

was added and the incubation period was 1 hour. The results of these experiments are given in Table 2. As was expected, high dopa decarboxylase activity was found in the kidney with an approximately 50% conversion of dopa to dopamine. However, no dopa decarboxylase activity could be demonstrated in any of the melanoma tissue even with addition of pyridoxal-5-phosphate.

3. Dopamine β -Oxidase Activity:

Spectrofluorimetric Assay: Three samples of melanoma tissue and two samples of liver (from previously frozen tissue) were assayed by the nonradioactive method for the presence of dopamine activity. With the melanoma tissue, the values of noradrenaline and adrenaline obtained from incubation of 500 μ g of dopamine are given in Table 3. This indicated a conversion of approximately 0.01% of the dopamine. No conversion was found after incubation with liver.

Radioactive Dopamine Assay: In these experiments both fresh and previously frozen tissue were assayed together with fresh hamster adrenal as a control. No dopamine β -oxidase activity could be demonstrated by this method either in the melanomas or the hamster adrenal. Addition of fumarate and catalase to the incubation mixture had no significant effects on the results.

the first part of the document, the author discusses the importance of the study and the objectives of the research. The author then proceeds to describe the methodology used in the study, including the data collection and analysis techniques. The results of the study are presented in the following section, and the author concludes with a discussion of the implications of the findings and suggestions for further research.

2. Literature Review

The literature review in this study focuses on the theoretical framework and the empirical research related to the topic. The author identifies the key concepts and theories that underpin the study and discusses the findings of previous research. The review highlights the gaps in the existing literature and justifies the need for the current study. The author also discusses the methodological approaches used in the literature and compares them with the approach used in the current study.

3. Methodology

The methodology section describes the research design and the data collection and analysis procedures. The author explains the choice of the research design and the sampling method used. The data collection methods, including the use of questionnaires and interviews, are described in detail. The data analysis techniques, including statistical analysis and content analysis, are also explained. The author discusses the strengths and limitations of the methodology used in the study.

Discussion

If catecholamine synthesis and storage occurred in the mammalian melanocyte, one would expect to find relatively high concentrations of catecholamines in the melanoma. The results of these experiments show amounts of catecholamines in the melanoma which are not particularly high and which are compatible to what has been found in the skin and other organs such as the brain and heart.

Neither of the two enzymes, dopa decarboxylase nor dopamine β -oxidase could be demonstrated in the melanoma. The dopamine β -oxidase experiments were inconclusive in that no adequate control could be established to show that the experimental method was not deficient, particularly in the light of the fact that the adrenal is known to contain dopamine β -oxidase activity. Dopa decarboxylase activity, however, was demonstrated in the kidney but not in the melanoma. One can thus assume that the enzyme is not present in the melanoma. In regard to this, it is interesting that recently Hakanson et al.⁴⁷ were able to demonstrate high dopa decarboxylase activity in hamster melanoma. This difference in findings cannot be explained, however it may be related to differences in the types of hamster melanoma tissue used.

On the basis of the above experiments, no evidence could be found to support the theory that catecholamines are synthesized or stored in mammalian melanocytes.

CONTENTS

If this book is to be of any use to you, it is essential that you should read it from cover to cover.

The first part of the book is devoted to the study of the general theory of the subject.

The second part is devoted to the study of the special theory of the subject.

The third part is devoted to the study of the applications of the subject.

The fourth part is devoted to the study of the history of the subject.

The author wishes to express his thanks to the following persons for their kind criticisms and suggestions:

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The author wishes to express his thanks to the following persons for their kind criticisms and suggestions:

Catecholamine Assay

<u>Tissue</u>		<u>Method</u>				
		<u>Single Standard</u>		<u>Standard Curve</u>		
		NA	A	NA	A	
Frozen Melanoma	1.	0.15	0.06	5.	0.21	-
	2.	0.11	0.05	6.	0.17	-
	3.	0.24	-	7.	0.25	-
	4.	0.09	0.009	8.	0.20	-
Fresh Melanoma	9.	0.17	0.0005			
	10.	0.15	0.004			
Frozen Liver	1.	0.04	-			
	2.	0.02	-			
	3.	0.03	-			
	4.	0.05	-			

Table 1.

Noradrenaline and adrenaline in hamster melanoma and liver ($\mu\text{g/g}$). The catecholamine content of both fresh and previously frozen hamster melanoma and liver (weighing 2-6 g) were determined by extraction and purification with Al_2O_3 (Anton and Sayre) and spectrofluorimetric measurement according to Axelrod's method based on the formation of fluorescent trihydroxyindole from the catecholamines.

Dopa Decarboxylase Assay

<u>Tissue</u>	<u>Radioactivity (CPM)</u>				
	<u>Dopa</u>	<u>Dopamine</u>	<u>NA</u>	<u>Origin</u>	<u>Random</u>
#1 Blank	8,955	70	120	185	50
Kidney	5,925	5,955	50	960	55
Melanoma	9,200	135	170	715	40
Melanoma	7,410	55	60	320	55
#2 Blank	5,595	50	60	380	85
Kidney	4,585	6,230	100	220	50
Melanoma	4,150	65	65	435	40
Melanoma	7,225	105	90	210	50
#3 Blank	3,955	55	75	585	35
Kidney	3,460	4,655	70	220	35
Melanoma	4,095	55	50	130	40
Melanoma	3,725	50	65	120	50

Table 2

Dopa decarboxylase activity in hamster melanoma and kidney. Activity in the tissues were assayed by incubation of dopa-2-C¹⁴ with 250 mg tissue in a nitrogen atmosphere at 37°C extraction of catecholamines by the method of Anton and Sayre, separation of the catecholamine by thin layer chromatography (Schneider and Gillis) and the radioactivity of the separate catecholamines measured in a liquid scintillation counter. Experimental group #1 contains fresh kidney and fresh melanoma tissue incubated for one hr. Group #2 contains fresh kidney and previously frozen melanoma, each incubated for 1 1/2 hrs. Group #3 is comprised of fresh kidney and previously frozen melanomas, each incubated for 1 hr. in the presence of 20 µg of pyridoxal-5-phosphate. The random sample was taken from an area through which the catecholamines did not pass.

2000-2001

2000-2001					Years
Year	2000	2001	2002	2003	
19	100	100	100	100	100
	100	100	100	100	100
	100	100	100	100	100
	100	100	100	100	100
20	100	100	100	100	100
	100	100	100	100	100
	100	100	100	100	100
	100	100	100	100	100
21	100	100	100	100	100
	100	100	100	100	100
	100	100	100	100	100
	100	100	100	100	100

T. 2000

The following table shows the results of the 2000-2001 survey. The data is presented in three columns, one for each year. The first column shows the number of respondents, the second column shows the percentage of respondents, and the third column shows the number of respondents who are currently employed. The data is presented in three rows, one for each year. The first row shows the number of respondents, the second row shows the percentage of respondents, and the third row shows the number of respondents who are currently employed.

Dopamine- β - Oxidase Assay

Melanoma Samples	<u>NA</u>	<u>A</u>
1.	2.15	0.2
2.	2.15	0.21
3.	1.75	0.18

Table 3

Dopamine- β -Oxidase Activity in Hamster Melanomas. The activity was measured by incubating 500 μg of dopamine with 250 mg of melanoma tissue in the presence of ascorbic acid, pyrogallol, and iproniazid 2 hours in air atmosphere at 37°C . The noradrenaline and adrenaline content of the incubation mixture was assayed by extraction and purification using Al_2O_3 (Anton and Sayre) and measured spectrofluorimetrically by the method of Axelrod.

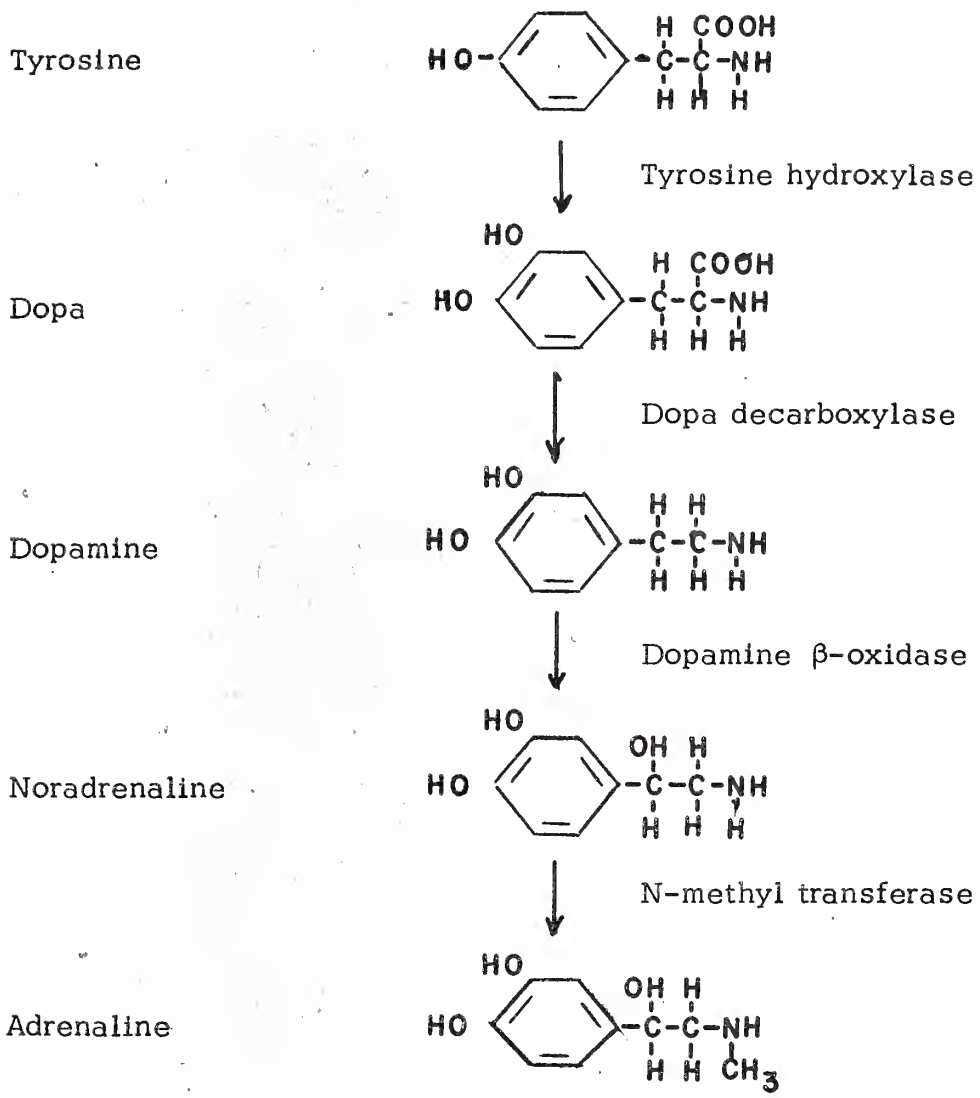
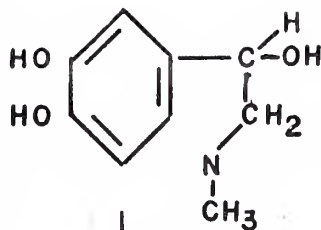


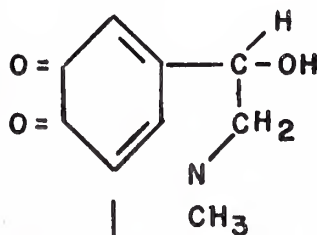
Fig. 1 Biosynthesis of Catecholamines

Adrenaline



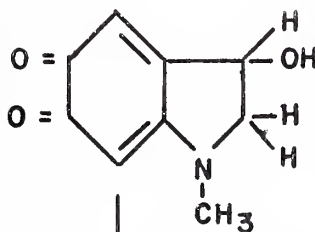
-2H

Adrenaline -
quinone



-2H

Adrenochrome



OH^- anaerobic

Adrenolutine
(3,5,6-trihydroxy-
1-methylindole)

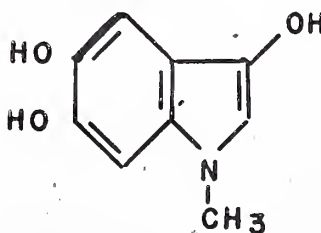


Fig.2. Conversion of adrenaline to florescent trihydroxyindole, adrenolutine.



Fig. 3. Hamster with subcutaneous melanoma three weeks after transplantation.



Fig. 4. Hamster with subcutaneous melanoma exposed.

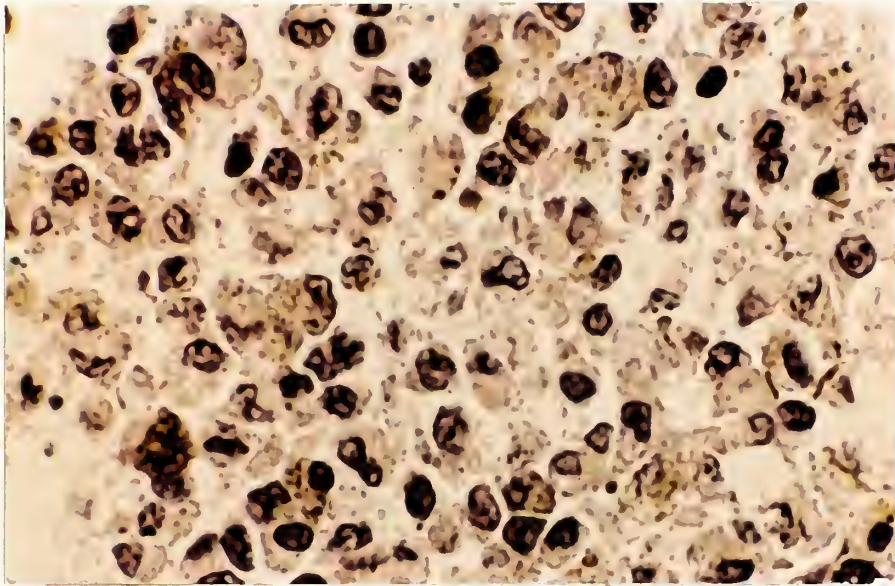


Fig. 5. Melanoma cells. Hematoxylin and eosin stain.
Magnification: x 590

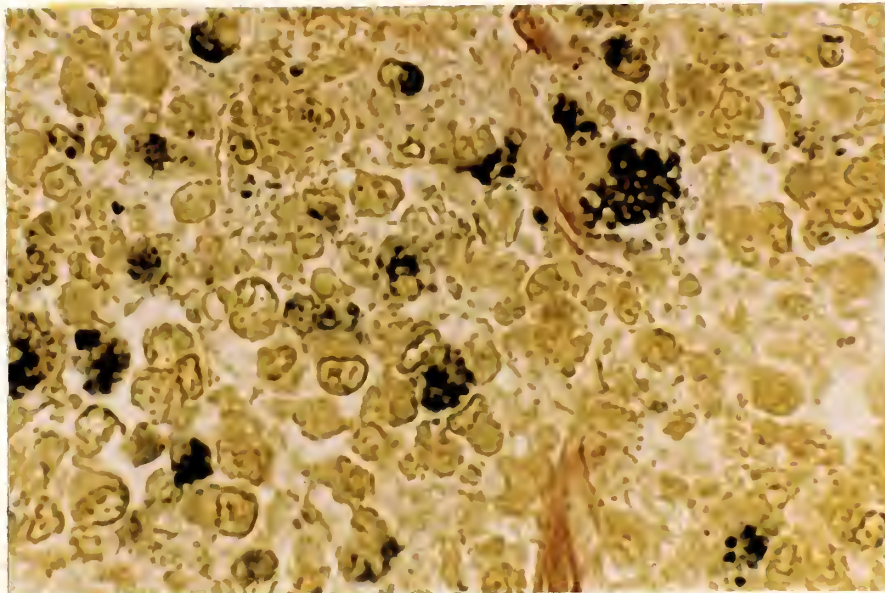


Fig. 6. Melanoma cells. Ferrous ion uptake stain.
Magnification: x 590



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