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ON CATECHOLAMINES IN MAMMALIAN MELANOCYTES

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Acknowledgements

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Introduction

In 1904 Elliott[1], on the basis of Lewandowsky's[2] 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 Euler[3] 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].

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 toads[7]. 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 gram[8].

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 tissue[9]. 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 increase[7].

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 tissue[5].

On the cellular level, the catecholamines are present in the chromaffin cells described and named by Henle[10] 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

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 cells[11].

More recently, Nordenstrom and Adams-Ray[12] 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 Philips[13] 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 Mercantini[14], Coupland and Heath[15], and Matz[16] 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 granules[16] and by electron microscopic studies of the adrenal medulla[17], showing the two separate cell granules.

The biosynthetic pathway for the catecholamines was initially postulated by Blaschko[18] 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 dihydroxyphenylalanine) 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 occuring in melanocytes is catalyzed by tyrosinase, an enzyme which requires copper for a cofactor[21]. Recent studies have demonstrated the presence of another enzyme, tyrosine hydroxylase, which catalyzes the same reaction in the adrenal medulla and in the brain[22]. 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 coenzyme[23]. 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 1938[24] and in the adrenals by Langeman in 1951[25]. The enzyme is found in the cell supernatant[26] and

requires the presence of pyridoxal-5-phosphate[27] 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 demonstrated[4].

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 derivatives[33]. 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:

$$\text{Dopamine} + \text{Ascorbate} + O_2 \xrightarrow{\text{Fumarate}} \begin{array}{l}\text{Noradrenaline} + \\ \text{Dehydroascorbate}\end{array}$$

However, the role of fumarate is still undetermined[34].

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 heart[35].

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 ganglia[31].

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.

The dispute over the claim of Nordenstrom and Adams-Ray that chromaffin cells exist in the mammalian skin has already been discussed. Recently Halvor Möller in his studies on catecholamines in the skin of mice, rats, rabbits and man was able to demonstrate the presence of catecholamines[36], a small but definite amount of which remained after treatment of the animals by sympathectomy or with reserpine[37], and the presence of both enzymes, dopa decarboxylase[38] and dopamine β -oxidase[32]. He was able to demonstrate the formation of noradrenaline from incubation of C^{14} dopa and C^{14} dopamine with skin, thus indicating that biosynthesis of the catecholamines may exist in the skin *in vivo*[39]. 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 metabolism[41]. Histologically, the chromaffin cell and the melanocyte are similar morphologically and share certain staining reactions, both being argyrophilic[11]. Biochemically, as already noted, melanogenesis

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.

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 1957[42]. 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-C^{14} with a specific activity of 5 mc/35mM and dopamine-2-C^{14} 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 Sayre[9], a modification of the method of Shaw[43]. The catecholamines were extracted from the tissue with acid, adsorbed on aluminum oxide and re-eluted with acid. The

catecholamine content of the eluate was determined spectrofluorometrically by the method of Axelrod[44], based on the trihydroxyindole method first described by Ehrlen[45]. 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 $HClO_4$, and transferred to a 50 ml beaker containing 400 mg Al_2O_3, 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 Al_2O_3 allowed to settle, the supernate aspirated and discarded. The Al_2O_3 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 $HClO_4$ is added to the precipitated Al_2O_3 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:

1. Blank (0.2 ml. 0.05 N $HClO_4$)
2. 200 mμg NA standard (in 0.2 ml 0.05N $HClO_4$)
3. 200 mμgNA standard (in 0.2 ml 0.05N $HClO_4$)
4. 200 mμg A standard (in 0.2 ml 0.05N $HClO_4$)
5. 200 mμg A standard (in 0.2 ml 0.05N $HClO_4$)
6. Eluate 0.2 ml
7. Eluate 0.2 ml
8. Eluate + 100 mμg NA (in 0.2 ml 0.05N $HClO_4$) (internal standard)
9. Eluate + 100 mμg 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 0.25% $K_3Fe(CN)6$ 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 $K_3Fe(CN)6$ 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 mμ and 505 mμ 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 mμg NA standard pH 7.0
b = Reading 100 mμg A standard pH 7.0

e = Reading 100 mμg NA standard pH 3.5
f = Reading 100 mμg A standard pH 3.5
s = Reading unknown at pH 7.0
t = Reading unknown at pH 3.5
c = Concentration NA standard =1 μg/ml
d = Concentration A standard =1 μg/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 μg/ml
A= concentration of A in eluate in μg/ml

Solving equation: $c + d = 1$

$A = (se - at)/(be - af)$ μg/ml
$NA = (s - bE)/a$ μg/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 mμg NA + 5 mμg A
2 - 10 mμg NA + 10 mμg A
3 - 15 mμg NA + 15 mμg A
4 - 25 mμg NA + 25 mμg 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

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

Dopa Decarboxylase Assay: Dopa-2-C^{14} 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 mμg dopa, 1 mg dopamine, and 1 mc dopa-2-C^{14} 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 $HCl0_4$. 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 $HCl0_4$. 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 Gillis[46] 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 H_2O was layered on glass plates at a thickness of 0.5 mm and air dried. 5 λ aliquots of the unknown were spotted with 1λ 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 $K_3Fe(CN)_6$ in 50 ml H_2O, 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

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 Möller[32].

Procedure:

250 mg homogenized melanoma tissue was incubated with 500 μg dopamine in the presence of 500 μg of pyrogallol and iproniazid and 200 μg 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 $HClO_4$. The solution was then assayed for the presence of catecholamines using the spectrofluorimetric method previously described.

The second method used the same incubation procedure with the exception that 1 mc of radioactive dopamine-2-C^{14} 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-C^{14} experiment.

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 Möller 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

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 µg 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.

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.

Catecholamine Assay

Tissue		Method				
		Single Standard			Standard Curve	
		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 (μg/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

	Tissue	Radioactivity (CPM)				
		Dopa	Dopamine	NA	Origin	Random
#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-C^{14} 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.

Dopamine- β - Oxidase Assay

Melanoma Samples	NA	A
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 mμg 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.

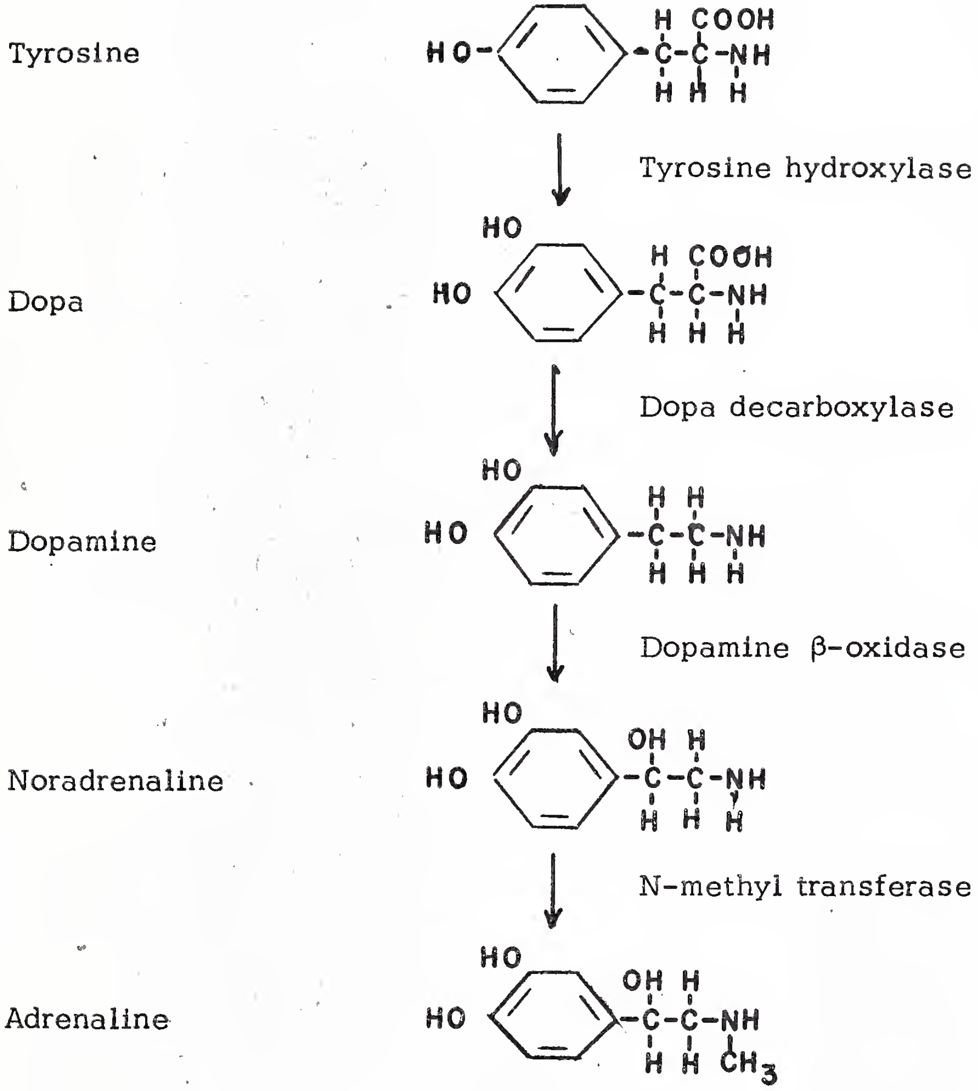

Fig. 1 Biosynthesis of Catecholamines

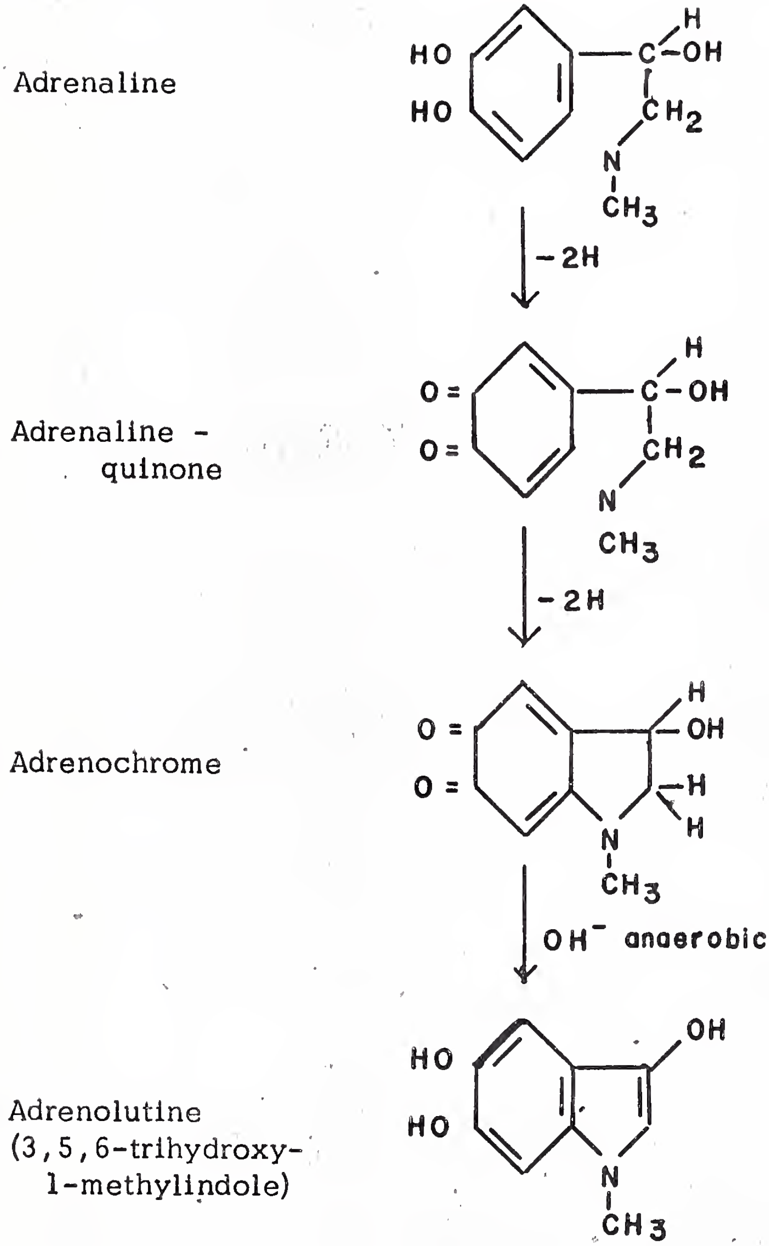

Fig.2. Conversion of adrenaline to florescent trihydroxyindole, adrenolutine.

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

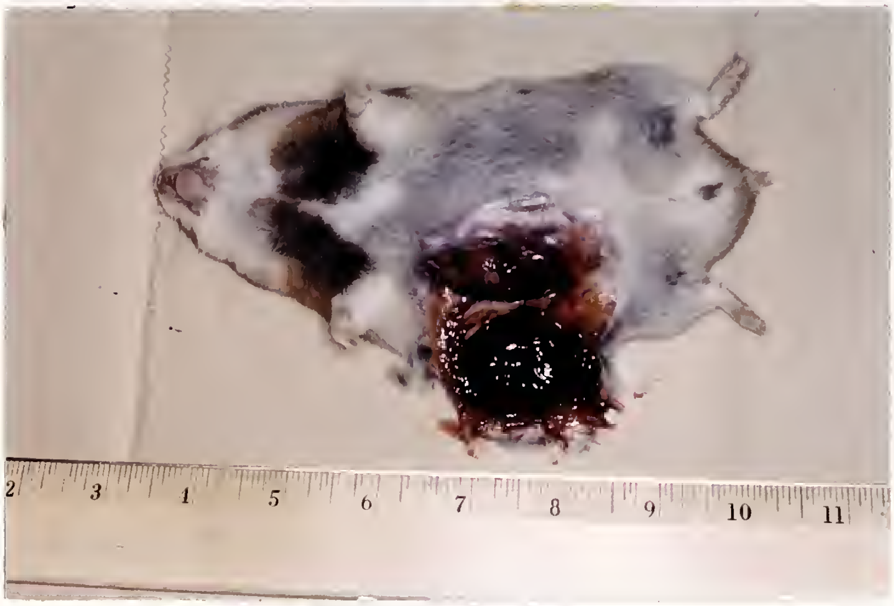

Fig. 4. Hamster with subcutaneous melanoma exposed.

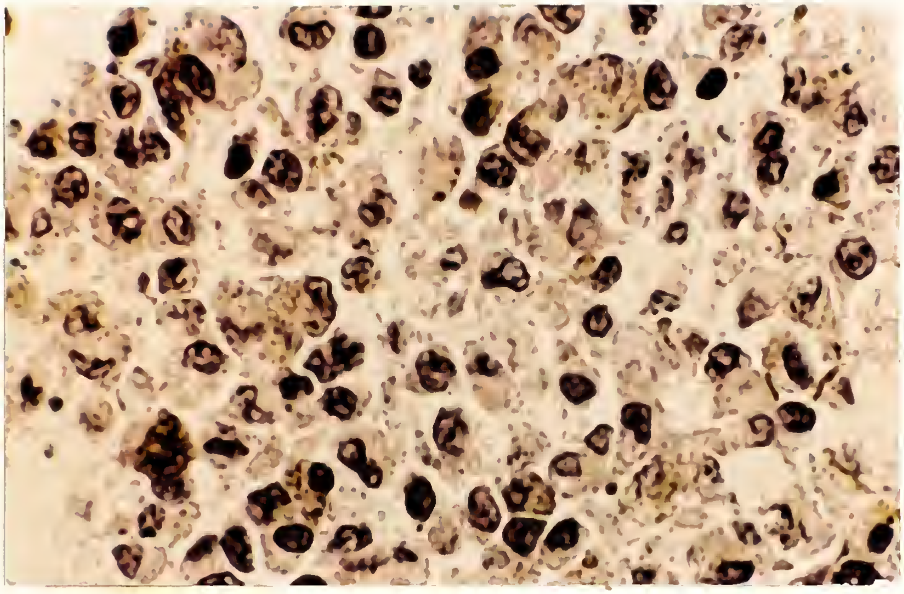

Fig. 5. Melanoma cells. Hematoxylin and eosin stain.
Magnification: x 590

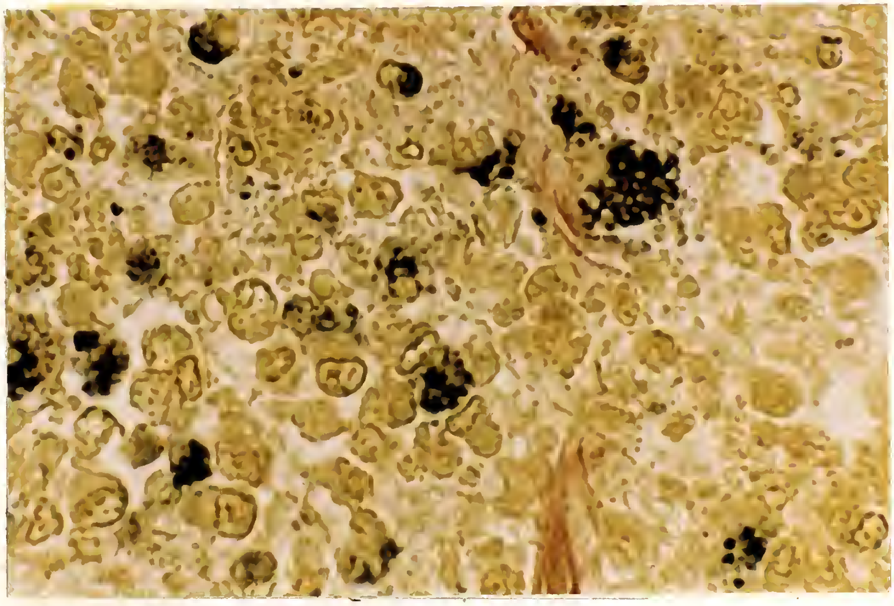

Fig. 6. Melanoma cells. Ferrous ion uptake stain.
Magnification: x 590

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