INTRODUCTION

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Historical Review :

The adrenal gland was first described by Eustachius in(1563). However, Leydig was the first in(1852), to differentiate in it between cortex and medulla.

The stainability of the adrenal medulla has been described by Vulpian (1856), who noticed a greenish colouration which took place when the adrenal medulla was moistened with a dilute solution of ferric chloride. It was thus concluded that a substance with a catechol nucleus may be secreted by the adrenal medulla.

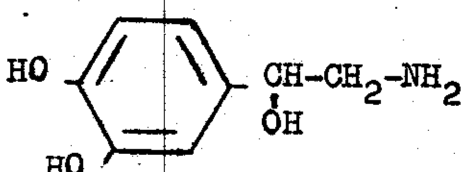
Oliver and Shaefer (1894) demonstrated the remarkable rise of blood pressure following the injection of adrenal medullary extract.

In the studies of Lewandowsky (1899 & 1900) and Langely (1901) attention was drawn to the striking similarity between the response to stimulation of the sympathetic nerves to an organ and the reaction in the corresponding organ to the injection of adrenal gland extract.

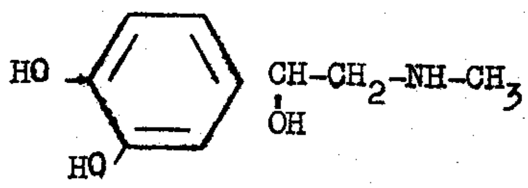
The final proof of the presence of noradrenaline concurrently with adrenaline in the adrenal gland of a number of animals, was given by several investigators Holtz and Schaumann(1948), Bulbring and Burn (1949), Euler and Hamberg (1949). It was, further from adrenal gland extract that Bergstrom et al. succeeded in isolating noradrenaline chemically in (1949). Later in (1950) and (1951) Euler gave a detailed account of the chemical and biological effects of such hormone.

Goldenberg (1951) stated that the sympathetic transmitter in adrenal medulla is noradrenaline or a mixture containing mostly noradrenaline and little adrenaline.

So, it was concluded that the adrenal medulla is concerned with the elaboration of still a second hormone "norepinephrine" or "noradrenaline" which is a primary amine of "adrenaline" or "epinephrine", representing a demethylated epinephrine (Soffer et al., 1961).



Noradrenaline



Adrenaline

Histogenesis :

In (1946), Cowdry mentioned that the two components of the adrenal gland i.e. cortex and medulla are different developmentally, histologically and functionally.

In lower vertebrates e.g. fishes, they are permenantly separated, but as we ascend the scale of animal life into the amphibia, reptiles and birds, they become closely associated.

In mammals, they finally unite to form a single glandular structure, in which the adrenal medulla is evidently shielded by the cortex. This arrangement has gradually came about in the evolution of vertebrates.

Grollaman (1947) in his book "Essentials of endocrinology" stated that the medulla is a part of a widely distributed chromaffin system.

The medullary cells are ectodermal in origin since they are derived from the neural crest in common with the sympathetic nerve cells. In the course of development unknown forces have caused them to occupy this central position.

In the eighth week of human foetal life (Gray, 1958), cells from the neighbouring masses of sympathochromaffin tissue migrate into the foetal cortex along the central vein and form the medullary portion of the gland.

These cells become differentiated into two types: the sympathoblasts leading to mature sympathetic ganglion cells and the pheochromoblasts which develop into the chromaffin cells.

Differentiation into chromaffin cells begins in the third month of foetal life but is not complete until birth. Although adrenaline is present as early as the third month of foetal life, the chromaffin reaction, however, is not obtained until the fifth month of intrauterine life.

In a comparative chemical study, Hokfelt (1951) found that the first appearance of catecholamines in albino rat was at the eighteenth day old foetus. Both adrenaline and noradrenaline could be identified with preponderance of noradrenaline (80%). The adrenaline content gradually increases to reach(84%)of the total catecholamines in 120 days old rats which was the oldest studied by Hokfelt.

However, the first appearance of catecholamines in man was at 28th weeks before birth and consisted only of noradrenaline. Adrenaline appeared about the 22nd weeks before birth and increases gradually in percentage to reach 93% at the senile age of 75 years.

The results of Roffi (1964), Brundin (1965) and Coupland and MacDougall (1966) in the rabbit's adrenal medulla indicated that by the twenty-eighth day of gestation, individual adrenal medullary cell granules stored either adrenaline or noradrenaline, while, at birth adrenaline comprised 80 - 90% of its catecholamines.

Coupland and Brenda (1968) in the same animal were able to identify cells with chromaffin granules in fourteen days-old embryos and all subsequent stages. The chromaffin granules had been identified in medullary cells which appeared to be intermediate in form between primitive sympathetic cells and pheochromoblasts. Such granules appeared, as well, in pheochromoblasts and pheochromocytes (chromaffin cells). The primitive sympathetic cells, however, do not exhibit such chromaffin granules. In the same year, the same authors stated, that some granules of adrenal medullary cells gradually increase in size after the eighteenth day of gestation. This increase was accompanied by a change in electron density, from high to moderate, and a change in the type of the stored amine from noradrenaline to adrenaline.

The average diameter of the granules of adrenomedullary cells continues to increase throughout the foetal period of rabbit and by twenty-eighth days it is possible to distinguish two distinct populations of adrenomedullary cells. The most numerous (80%) had a larger diameter and are the adrenaline storing cells, while the remainder are smaller ones and are the noradrenaline storing cells, both adrenaline and noradrenaline storing chromaffin cells are identified at birth.

Although the granules in a section through a particular cell were mostly either of adrenaline or noradrenaline type, however, the two types of granules may occasionally be seen in a single cell.

Such granules were also found to increase in diameter throughout the period between birth and the age of three months (Coupland and Brenda, 1970).

The discovery of two different chromaffin cells in the adrenal medulla :

It was first thought that the frequently observed patches in chromaffin reaction were due to groups of cells in various phases of their secretory cycle (Bennett, 1941). However, Holtz et al. (1947) referred this patchiness to the presence of another hormone probably noradrenaline. This was confirmed, in (1948), by the investigation of Holtz and Schaumann and Euler & Hamberg (1949 b).

This discovery of noradrenaline aroused the search for the different cell types in the adrenal medulla and the study of whether the medullary hormones were released independantly or were secreted as a mixture of constant proportion. Bergstrom et al. (1954).

The localization of the two catecholamines in two different adrenomedullary cells was proved by the studies of Burn et al. (1950), Hokfelt (1951) ; Outschoorn (1951), Eranko (1952) in the rat and the cat. They found that only the adrenaline content can be experimentally decreased by insulin without affecting the noradrenaline content.

Eranko (1951 & 1955) employing various staining techniques succeeded in demonstrating the presence of the two kinds of chromaffin cells within the adrenal medulla of the rat and cat :

A) In staining for acid phosphatase, he found that the majority of the adrenal medullary cells contained acid phosphatase. Inbetween such positive cells groups of acid phosphatase negative cells were present.

B) When adrenal medullary tissue was fixed in formalin and inspected under ultraviolet light, some cell groups were brightly fluorescent against a background of faintly fluorescent cells.

C) Immersion of the medullary tissue in ammoniacal silver nitrate resulted in darkening of only those cells which fluoresced when fixed in formalin, while most of the cells remained unstained.

At that time, Hillarp and Hokfelt (1954) described an additional technique to distinguish between the two types of cells employing potassium iodate, in which brown staining of groups of cells were seen against a yellowish background, and were identical in distribution, size and shape, to the fluorescing cell-islets.

Brücke , et al. (1952), Redgate and Gellhorn (1953), found that stimulation of restricted areas in the hypothalamus may selectively increases the adrenaline or noradrenaline content of blood taken from the adrenal vein.

The further investigations of Hokfelt (1953), Hillarp and Hokfelt (1954) and Eranko (1954) in the rat and the cat were all in favour of the assumption that adrenaline and noradrenaline of the adrenal medulla are two independant hormones which are selectively made use of under different conditions.

Hillarp and Hokfelt (1954), as well, found that the number of medullary cells containing noradrenaline and which form brown insoluble pigments after potassium iodate oxidation varies more or less proportionately with the content of noradrenaline.

Eranko & Hanninen(1960) found that nicotine injection in the rat induced a selective loss of noradrenaline and selective disappearance of the chromaffin reaction and of the fluorescence from the medullary islets of adrenal medulla.

Later, Wood (1963) described another staining method with eosin yellow and aniline blue, which showed most of the cells to be brown to purple in colour and the rest yellow in colour. This latter method had shown that there exist morphological differences between the two cell types on the basis of granularity and size.

The majority of cells appeared to be larger in size with finely granular cytoplasm, while, the minority were smaller in size with a larger more densely granular cytoplasm. The cortex appeared blue in colour.

To sum up, these previous studies suggested that those cells of the adrenal medulla which stained darkly with ammoniacal silver nitrate, brown with potassium iodate or yellow with Wood's stain and which lacked acid phosphatase and fluoresced when fixed in formalin, were concerned with elaboration of noradrenaline; while, cells which stained lightly with silver nitrate, yellow with potassium iodate and purple with Wood's stain, containing acid phosphatase and which did not fluoresce, were adrenaline secreting cells.

Such studies, strongly suggested that the adreno-medullary cells secreting adrenaline and those secreting noradrenaline, inspite of their similarity and close topographical relationship were fairly autonomous in function.

The physiological value of the localization of the two catecholamines in two different kinds of cells in the adrenal medulla lies in the fact that either of which can be secreted independantly to meet the specific requirements of the organism.

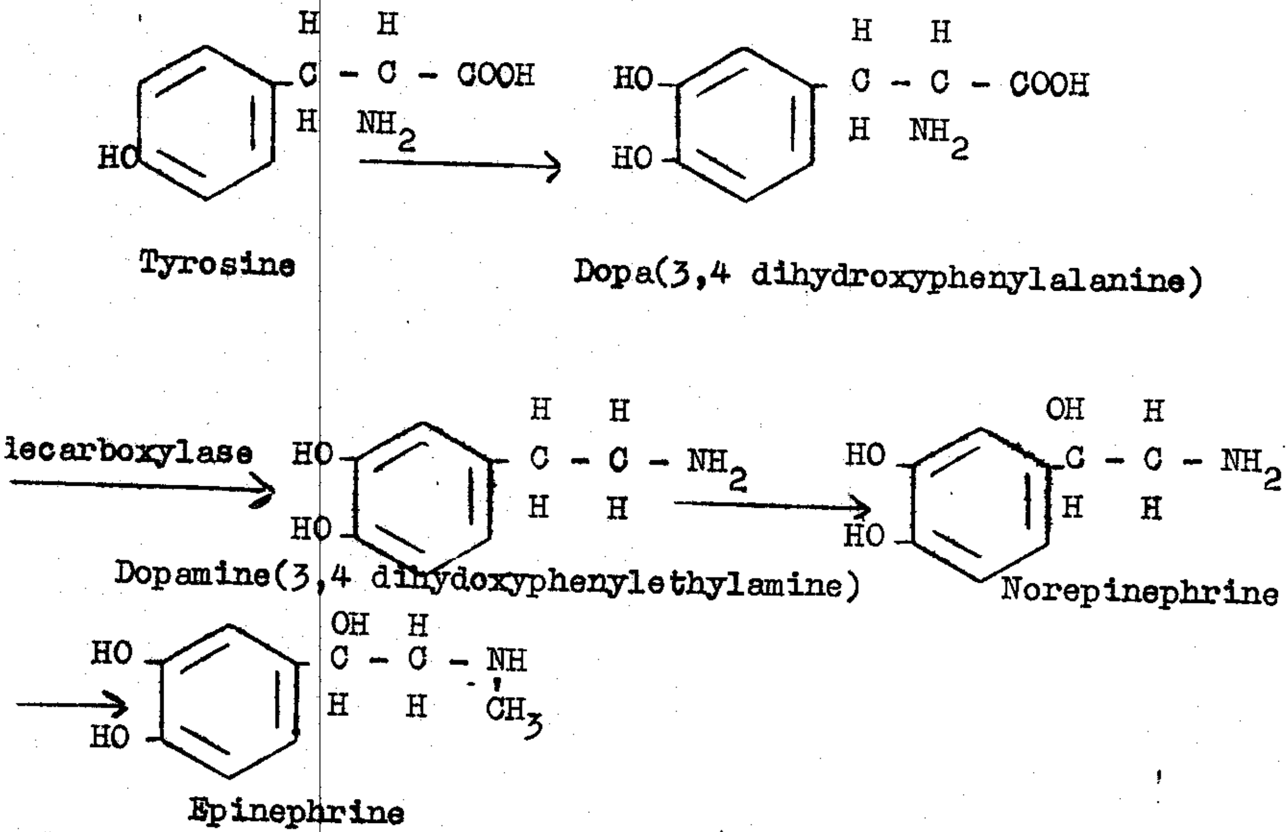
Adrenaline and noradrenaline hormones :

Biosynthesis :

Gurin and Delluva (1947) and Udenfriend et al. (1953) have demonstrated that both phenylalanine and tyrosine are in vivo the precursors of adrenaline.

Phenylalanine is oxidized to tyrosine, the latter is transported into the adrenal medullary cell to be oxidized to dihydroxyphenylalanine (dopa) in the non particulate cytoplasm, then it is decarboxylated to dihydroxyphenylethylamine (dopamine). The subsequent reactions involving dopamine apparently took place in the granules or mitochondria of the medullary cells (Soffer et al., 1961). At this site a good part of the dopamine is converted to dihydroxyphenyl-acetic acid, while a small proportion of the dopamine is hydroxylated to noradrenaline. Some of the noradrenaline undergoes methylation to adrenaline, both compounds are either stored in the granules of their secreting cells or secreted into the nonparticulate cytoplasm to the blood.

According to Blaschko (1959), the biosynthesis of adrenaline and noradrenaline occurs as follows :



<u>Sources</u> :

The nerve fibres are the important source of noradrenaline in the body (Bell et al., 1963), also the wall of blood vessels are rich in it.

While the important source of adrenaline hormone is the adrenal medulla. The ratio of adrenaline to noradrenaline in the human adrenal medulla is 4 : 1 (Wiggers, 1949).

Control of secretion :

Folkow and Euler (1949) have found that stimulation of one particular area of the hypothalamus causes mainly adrenaline to appear in the effluent, whereas stimulation of another area of the hypothalamus releases mainly nor-adrenaline. Stewart and Rogoff (1929) found that the average rate of liberation of adrenaline lies between 0.0001 - 0.0003 mgm per kgm body weight per minute with an average of 0.00025 mgm.

Keele and Neil (1960) stated that there are centres in the reticular formation in the medulla oblongata and hypothelamus which exercise a higher control.

Physiological actions :

Elmadjian et al. (1958) stated that active, agressive, emotional displays are related to increased excretion of noradrenaline, whereas tense anxious, but passive emotional displays are related to increased excretion of adrenaline in the urine. Keele and Neil (1960) stated that, the sympathetic nerves through their liberated noradrenaline regulate circulation, while the adrenal medulla with its [illegible] of adrenaline secretion, takes part in reac- [illegible] stress, exercise, exposure to cold and hypoglycaemia.

Best and Taylor (1966) stated that there is, also, a relationship between the adrenal medullary content of noradrenaline and the typical behavior in different species. Aggressive animals (cat, lion) have large amounts of noradrenaline in the adrenal medulla, whereas, timid animals (rabbit, guinea pig) have little.

Campbell et al. (1968) in comparing the biological activity of noradrenaline and adrenaline stated that : adrenaline causes excitatory responses in some regions and inhibitory ones in others. It excites constriction of the blood vessels of the skin, it stimulates piloerection and dilatation of pupil and in large quantities may cause sweating, it induces tachycardia, the cardiac output increases and elevate the systolic blood pressure, but not the diastolic one, the peripheral resistance falls, this is because adrenaline inhibits the smooth muscle fibres of all arterioles except those of the skin. It relaxes the smooth muscles of the uterus (in man), the bronchioles, the intestine and the bladder.

Adrenaline, also, has a metabolic effects, it produces a rise of the blood sugar concentration by stimulat- the breakdown of liver glycogen to glucose and it increases the metabolic rate.

On the other hand, noradrenaline has predominantly excitatory effects on the arteriolar musculature. It leads to a generalized vasoconstriction so that both systolic and diastolic blood pressures rises sharply and the peripheral resistance is greatly increased, bradycardia occurs and the cardiac output remains unchanged. Noradrenaline is approximately eight times weaker than adrenaline in causing hepatic glycogen breakdown.

The chromaffin reaction :

The granules in the adrenomedullary cells present characteristic staining reactions with various chemical agents. They are coloured black with osmic acid, green with ferric chloride and brown with chromic acid and its salts.

The latter one was termed "chromaffin reaction".

Gerard et al. (1930), stated that the so-called chromaffin reaction did not depend on chromium per se, but depended on the oxidation of certain organic compounds in the adrenal medulla to form brown polymers and that this oxidation can be produced by other oxidizing agents.

Hillarp and Hokfelt, in (1954), stated that for cytological demonstration of adrenal medullary catechols by oxidative - pigment formation, the following three criteria must be fulfilled :

1) The catechols must occur in sufficiently high concentration so that pigments can be formed.
2) The catechols must occur in such a state as to permit [illegible]tion.

[illegible]e catechols must be dissolved from the cells before pigments can occur.

Histology of the adrenal medulla :

The chromaffin cells of the adrenal medulla differ in their arrangement in various animal species.

In the common laboratory animal, albino rat, used in this work, the adrenomedullary cells are irregularly scattered (Grollman, 1947) and vary in shape from cylindrical to irregular polyhedral forms with a large number of blood sinusoids allowing intimate contact between the cells and the blood stream (Swinyard, 1943).

The cytoplasm of the medullary cells is distinctly granular. These granules which are chromophilic are never found in the cortical cells.

The number of chromaffin granules in the medullary cells is an index of their secretory content (Bailey, 1948). In depletion of the adrenal medulla, the granules disappear and are reformed again with storage of secretion. These granules are concerned with elaboration of adrenaline and noradrenaline hormones.

Lever in (1955) was the first to report on the ultrastructure of the adrenal medulla in the rat, followed later by Sjostrand and Wetztein (1956 & 1957) and by De Robertis and Vaz Ferreira in (1957), when they studied the adrenals of the mouse, guinea pig, cat and rabbit.

All the previous investigators observed small osmiophilic granules in the cytoplasm of the secretory medullary cells. The electron microscopic studies confirmed that the adrenomedullary chromaffin cells were essentially of two types, a dark larger cell containing abundant osmiophile adrenaline secretory granules which readily stained black with osmic acid against an opaque background cytoplasm and a smaller bright cell containing fewer number of secretory granules of noradrenaline.

The mitochondria are normally compact in the dark cells and often vaouolated in the light ones. Great variations in appearance of adrenaline granules had suggested their inception within saccular investments by an aggregation of micro-granules.

The medullary Golgi apparatus though frequently seen as a compact paranuclear complex may be more diffuse. The nature of some cytoplasmic sacs was equivocal, though many can be positively identified as ergastoplasmic, golgiform or mitochondrial in origin.

The chromaffin cells in man are large, ovoid columnar cells Cruickshank et al. (1968) arranged in rounded groups or in short cords around sinusoidal venules. Very fine brown granules can be seen in the cytoplasm of these cells if the tissue is fixed in chrome salts.

Sympathetic ganglion cells innervated by preganglionic sympathetic fibres are arranged singly or in small groups among the chromaffin cells of the adrenal medulla.

Small cells resembling lymphocytes, with deeply staining nuclei and little cytoplasm "the sympathogonia" are also present.

<u>Blood supply</u> :

The blood that reaches the adrenal medulla is mainly venous with little arterial supply (Cowdry, 1946).

The blood is filtered through the cortex, so as harmful substances may have been partly or completely removed, perhaps others useful to the medulla have been added.

Certainly this blood is especially rich in corticosterones and may posses much vitamin C.

The capillaries in the medulla anastomose and run in all directions. They are wider than in the cortex, so can properly be termed sinusoids and are drained by thin walled venules.

All blood leaves at the hilus by a single adrenal vein in the wall of which much connective tissue and numerous smooth muscle fibres are arranged mainly in longitudinal bundles.

Lymphatic capillaries are not well developed. Some begin in association with the veins of the medulla and become confluent forming vessels that leave with the veins.

Numerous nerve fibres enter the medulla through the hilus. Most of these end in touch with the chromaffin cells but few pass out into the zona reticularis of the cortex.

<u>Comparative biological study of man and rat adrenals</u> :

According to Arai (1920), sixty-five days is the mean age of the first ovulation in rat, although according to Vierard's table the first menstruation in man appears between 8 - 10 years.

The cessation of breeding period in female rats takes place between 18 and 20 months, while in man between 40 to 45 years of age. Jackson (1913).

The period of gestation in albino rats is twenty-one days. The adrenals of the foetus are very small. During the last day of foetal life, they are about 0.5 mgm/pair, as was shown by Hokfelt in (1951), who carried out an exhaustive biological study of the catechol content in rat and man in different age groups.

As the catechol contents were very small, the adrenal glands of about thirty foetuses were required for each extract.

The catechol contents of the adrenals had been determined biologically and where sufficient material was obtainable the calorimetric method was also applied. The first amount of catechol content was found in rat's medulla at eighteen days of foetal life, it was about 0.015 µ/pair and 20% of which was adrenaline. At this age scattered chromaffin cells could be observed in the adrenal medulla.

From day to day thereafter both adrenaline and nor-adrenaline contents increased rapidly with preponderance of the methylated component "adrenaline" which reached

34% and 43% two and one day before birth respectively, while two days after birth adrenaline content was 56% and four days later reaches 60%. Thirteen days after birth it was 64% to reach 84% at 120 days old albino rat which was the oldest age studied by Hokfelt (1951).

The following table was given by Hokfelt to compare the adrenaline and noradrenaline contents in male and female rats :

	Male	Female
Noradrenaline (micro gm/gm tissue)	164.9	126.6
Adrenaline (micro gm/gm tissue)	899.0	787.0
Noradrenaline (micro gm/kgm body weight)	27.5	31.2
Adrenaline (micro gm/kgm body weight)	148.0	195.6

Although noradrenaline and adrenaline contents in micro gm/gm tissue, was higher in male than female, the amount related to kgm body weight was higher in female than male.

However, adrenaline content expressed in percentage of the catechols showed no significant difference between the two sexes.

There was no significant difference in catechol contents of adrenals during twenty-four hours.

When adrenaline and noradrenaline contents of the adrenals were studied in man , Hokfelt showed that since 28 weeks before birth till 75 years of age there was a gradual increase of the percentage of adrenaline from 0 - 93 %.

In the same year (1951) West and Hunter assorted that the sympathomimetic catechol compounds in man first appeared in the non methylated form noradrenaline. This was in contrast to foetal rat in which adrenaline and noradrenaline appeared concurrently three days before birth.

However, in both rat and man adrenaline becomes larger in percentage, in rats two days after birth and in man two years after birth.

Effect of unilateral adrenalectomy :

Several studies were made on unilateral adrenalectomy most of them dealed with its effect on the adrenal with no attention to its effect on the medulla.

Mac Kay et al. (1926) stated that the remaining gland hypertrophied and the increase in size was due to the increase in the size of the cortical cells. However, Pellegrino et al. (1963) studied cytochemically and physiologically the effect of monoadrenalectomy on the remaining gland, after different times from the operation ranging from 3 - 60 days and stated that the hypertrophy of the cortex was due to increase in the number of cells and not the cell volume. Although the nuclear volume increased in the first ten days, it later underwent a rapid decrease, returning after two weeks of the operation to the normal value.

The choice of the removal of left or right adrenal gland did not alter the results, as there was no difference according to the side of the removed gland.