II

HORMONAL AND DRUG FACTORS AFFECTING THE CATECHOLAMINE CONTENT OF RAT TISSUES

by

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THESIS

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A B B R E V I A T I O N S

N	noradrenaline
A	adrenaline
D	dopamine
5HT	5 hydroxytryptamine
gr.	gram
μg.	microgram
DCA	desoxy-cortico-steron-acetate
AX	adrenalectomy
TCA	trichloracetic-acid
BW	body-weight
SC	subcutaneously
MAO	mono-amine oxydase
ATP	adenosine triphosphate
CNS	central nervous system
A-V	atrio-ventricular
LSD	lysergic acid diethyl amid
CA	catecholamine

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INTRODUCTION AND HISTORICAL

1. INTRODUCTION

The marked electrolyte and plasma volume changes which occur after adrenalectomy do not satisfactorily explain either the death which occurs if treatment is not administered nor the pathological findings at autopsy. For example, normal animals can sustain an experimentally induced elevation of plasma potassium several milliequivalents greater than that seen in adrenal insufficiency or a reduction in blood volume of considerably greater than that occurring in this condition (1). The congestion of the mucosa of the gastrointestinal tract characteristic of adrenal insufficiency is seen also in animals dying of surgical shock (2). The circulatory shock seen in some animals dying a few days after the operation of adrenalectomy was investigated thoroughly by Swingle and Parkins (3) who found that healthy extract maintained adrenalectomized dogs were easily precipitated into shock by procedures well withstood by dogs with intact adrenals. A continuous adrenalin infusion was found to produce a shock-like picture in normal dogs as well as a pathological picture in the gastrointestinal tract like that after death following adrenalectomy (4).

While death from adrenal insufficiency, as indeed in many other instances, is associated with a decline in

cardiovascular function, there is evidence that there is something pathognomic about this following adrenalectomy. Evidence presented in 1914 by T.R. Elliott described a progressive failure of the blood pressure to respond to sympathetic nerve stimulation or to pressor drugs in cats dying of adrenal insufficiency (5). Little attention was paid to this work until it was repeated some 25 years later by Cleghorn (6). He suggested that this apparent decline in sympathetic nerve function gave rise to parasympathetic preponderance and described in detail the bradycardia seen in adrenal insufficiency, a finding noted many years earlier by Rogoff and Stewart among others (7). Furthermore, Hall and Cleghorn actually found myocardial infarction in some dogs dying of adrenal insufficiency. They also investigated the cardiac dysrhythmia which they observed in many such animals in a state of adrenal insufficiency (2).

A sober scientific approach should not preclude a scientific interest in phenomena which bear the forbidding aura of the mystical. Hence the study of voodoo death by so reputable a scientist as Walter Cannon makes it at least respectable for one working in a psychiatric institute to pay attention to such an obscure condition. In his paper published in 1942, Cannon gave many instances of mysterious, sudden, apparently psychogenically caused deaths. He made a thorough search of reports from many primitive societies before he convinced himself of the existence of voodoo deaths. He concluded that in this phenomenon the same elements of the nervous and endocrine systems were brought

into action, namely the sympatheticoadrenal, as in his experimental observations on rage and fear in cats. This in an area of observation very closely allied to that which has been discussed earlier in the present paper. Another scientist of repute, namely Curt Richter of Baltimore (8) has also been interested in sudden death following stress in wild and domesticated rats. He believed that over-activity of the parasympathetic system was involved in such animals with terminal slowing of the heart rate, lowering of respiration and lowering of body temperature, the stopping of the heart in diastole, as it does incidentally in adrenal insufficiency. It would seem that in this circumstance if there is a parasympathetic preponderance, but that it is largely due to the over-activity of this system per se rather than by a decrease in adrenergic activity.

To return now to the consideration of the effects of adrenalectomy, we see that this deprives animals of the adrenalin secreting medulla, not a serious matter in itself.

A. APPROACH TO THE PROBLEM

The work to be presented in this thesis attempts to elucidate the role which the adrenal glands exert on the catecholamine content of the rat heart. In the course of this investigation attempts were made to correlate the action of certain agents, primarily reserpine, but also dietary sodium with the catecholamine content of the rat

heart in presence and absence of adrenal glands. The opportunity was also taken to establish the effects of these various procedures on the catecholamines of the brain. The effect of adrenalectomy on urinary excretion of catecholamines was also determined.

II. HISTORICAL REVIEW

A. Identification of Catecholamines

Evidence for the chemical transmission or for a neuro-humoral mechanism goes back to Claude Bernard (9) who observed in 1876 that fatigue and the block produced by curare were localized at the junction between the nerve fibers and excitable cells. In 1877, DuBois-Reymond stated, that transmission of the nerve impulse may be produced either electrically by action currents or chemically by exciting substances like ammonia or lactic acid formed at the surface of nerve endings. It was recognized that some of the actions of the autonomic nervous system could be duplicated by drugs, such as muscarine which, applied to the frog heart, caused a slowing of the rate very similar to that produced by stimulation of the vagus nerve.

In 1895 Oliver and Schafer (10) described the pressor activity of an extract from the suprarenal gland and Langley (11) demonstrated the similarity of the extract to that evoked by stimulation of the sympathetic nervous system.

T.R. Elliott(12) postulated in 1905 that sympathetic nerve impulses released minute amounts of an epinephrine-like substance in immediate contact with effector cells. He was also impressed by the fact, that long after the sympathetic nerves degenerated the effector organs still responded characteristically to the hormone of adrenal medulla. Langley suggested that the effector cells have excitatory and inhibitory receptive substances, and that the response to epinephrine depended on which type of substance was present. It was considered at that time, that the para-sympathetic system released a substance similar to muscarine but proof was lacking.

The brilliant researches of Otto Loewi, began in 1921, established the first real proof of chemical mediation of nerve impulses, by the peripheral release of specific chemical substances (13). He stimulated the vagus nerve of a perfused frog heart (donor) and allowed the perfusion fluid to come in contact with a second (recipient) frog heart used as a test object. A substance was liberated from the first organ which slowed the rate of the second. He also discovered, that an accelerator substance similar to adrenaline was liberated into the perfusion-fluid when action of the sympathetic fibers in the frog's vagus predominated over that of inhibitory fibers. In 1927, Rylant duplicated Loewi's frog experiments using perfused rabbit hearts, Feldberg and Krayer cat and dog hearts. Due to the extensive investigation

by H.H. Dale, acetylcholine was identified as a likely chemical transmitter substance of the parasympathetic system as early as 1901-1904. In his paper with Barger in 1910 (14) he had also realized the significance of apparently small deviations in the actions of adrenaline from those obtained by stimulation of sympathetic nerves.

The properties of the "Acceleranzstoff" released by sympathetic nerve stimulation in the frog's heart observed by Loewi in 1921, seemed to point to adrenaline as the adrenergic transmitter, but other observations made the general validity of this assumption doubtful. In the same year Cannon and Uridil (15) reported that the liver, upon stimulation of sympathetic hepatic nerves, released an adrenaline-like substance which increased the heart rate and blood pressure, but did not dilate the pupil of the dog. Subsequent experiments by Cannon and co-workers firmly established, that this substance was a chemical mediator liberated by sympathetic nerve impulses at the neuroeffector junctions. The mediator was originally called "sympathin" by Cannon in order to avoid premature implications regarding its chemical nature. They concluded, that the mediator is liberated not only under experimental conditions, but also physiologically in the intact animal during struggling or excitement. It was likewise released during sham-rage in decorticate animals (16, 17). In many respects Cannon's

"sympathin" closely resembled adrenaline but the two substances differ in certain ways. Perhaps the greatest contrast between "sympathin" and adrenaline was seen in their action after ergotoxin. Whereas the ergot alkaloids reversed the rise in blood pressure produced by adrenaline they only reduced that caused by hepatic sympathetic stimulation.

As mentioned above, Dale and Barger had noted as early as 1911, that the effects of sympathetic nerve stimulation were more closely reproduced by the injection of primary sympathomimetic amines than that of adrenaline or other secondary amines. The possibility that demethylated adrenaline might be the "sympathin" had been repeatedly advanced by Melville, 1937 (18), Stehle and Ellsworth, 1937 (19), Melville and Oldham, 1936 (20), but definite evidence for its role as one of the sympathetic nerve mediators was not obtained until suitable chemical and biological assay methods were developed for the quantitative determination of small amounts of sympathomimetic amines in extracts of tissues and body fluids. Although recognition of noradrenaline as a naturally occurring substance of high biological significance was only achieved within the last 15 years, the substance itself had been known for a considerable time. Noradrenaline was synthesized in 1904 by Stolz (21) who noticed, that it was less toxic than adrenaline. In 1933 Cannon and Rosenbluth postulated the existence of two forms of "sympathin",

"sympathin E", (excitatory), and "sympathin I", (inhibitory) (22). Cannon's original theory, that the same chemical mediator "M" is released at nerve endings and that it is combined with the hypothetical substance "I" or "E", has only historical significance. Bacq, working in Cannon's laboratory in 1934 suggested, that "sympathin I" of Cannon was identical with adrenaline, and "sympathin E" with noradrenaline (23). Cannon and Lissak (24) in 1939 found, that cat hearts contained a sympathomimetic substance which they concluded to be adrenaline. Shaw (25) found that mammalian heart extracts contained an adrenaline-like substance which gave an arsenomolybdate color reaction. Raab repeating Shaw experiments (26) with extracts of rat and human heart found that they contained another catechol in addition to adrenaline. The proof that noradrenaline was actually a specific factor of the sympathetic nerves was provided by experiments of U.S. von Euler, 1946-48 (27) . He showed that extracts of such nerves and of organs supplied by them contained appreciable amount of noradrenaline, specifically the levo-form (28). Using the extracts prepared from cattle hearts he demonstrated the presence of a sympathomimetic compound which differed from adrenaline and which had the characteristic of noradrenaline (29). Goodall in 1951 (30) determined the quantity of adrenaline and noradrenaline in cattle hearts, and subsequently Holtz, Kroneberg and Schumann (31), Hokfelt (32), also demonstrated the presence of

adrenaline and noradrenaline in mammalian hearts.

Another line leading to recognition of noradrenaline as a biologically important substance was followed by Holtz and his co-workers (33), partly as a logical consequence of the discovery in 1938 of the enzyme l-dopa-decarboxylase in mammalian tissues, which converted dopa (3-4, dihydroxyphenylalanine) to hydroxytyramine (dopamine), which differs from noradrenaline only by a side-chain hydroxy-group. That noradrenaline might be formed during biosynthesis of adrenaline originating from dopa was postulated by Blaschko as early as 1939 (34). It is accepted now, that noradrenaline serves as a sympathomimetic neuro-transmitter substance and adrenaline as an adrenal medullary hormone. However, a precursor, or possibly an additional neurohormone, hydroxytyramine (dopamine), can also be detected in appreciable quantities in nerves and certain innervated organs (35, 36, 37).

B. Measurement of Catecholamines

In the quantitative estimation of catecholamines in secretions and body fluids and organs two principal problems are encountered. Firstly, the disturbing action of impurities of different kinds, have to be considered and the impurities if necessary removed. Secondly, since the extracts or biological fluids used for catecholamine assay nearly always contain different catecholamines in a variable mixture it is necessary either to separate them before assay or to use methods which permits the assay of each of them in a mixture.

a) Preparation of the Extracts for Estimation of Catecholamines

Acid ethanol or trichloracetic acid are generally used. For most organs 10% trichloracetic acid is a suitable extraction medium if the tissue is finely minced. The organ, if intact, can usually be left for several hours at room temperature before extraction without inactivation of its catecholamines, but then disintegration and inactivation set in rapidly unless an extraction medium is present which will inhibit enzymatic destruction (38). For the same reason frozen organs generally must not be allowed to thaw before extraction, since catechol inactivation occurs even in an apparently intact organ.

b) Purification Methods

Attempts have been made to purify crude extracts by dialysis (Loewi 1936) (39) (Pekkarinnen 1948) (40) or by removal of disturbing agents with the aid of adsorption on various earths. A method of purification which has proved most useful is adsorption on aluminium hydroxyde, described by Shaw in 1938 (41), or on aluminium oxide. These adsorption agents, especially the aluminium hydroxyde, show a high degree of specificity for catechol derivatives, which are adsorbed easily and completely. Adsorption can be attained by adding freshly prepared aluminium hydroxyde to the extract and adjusting the pH to 8 at which the adsorption is complete.

Practically no adsorption takes place at pH 4, and the adsorption is highly specific, since monohydroxyphenyl derivatives are not adsorbed to any appreciable degree. Of other substances ascorbic acid is adsorbed to some extent (42) which may enhance the destruction of catecholamines at a pH of approximately 4.

The catecholamines can be eluted from the aluminium oxide with the aid of acids such as acetic or tartaric, but also with mineral acids (43, 44). The recovery of adrenaline and noradrenaline from aluminium hydroxyde is about 70-75%, from aluminium oxide 80-90% (45).

c) Separation of Catecholamines

Filter paper chromatography can be adopted to serve a preparative purpose prior to assay. The rate of flow of a very small amount of active substance in paper chromatography can be determined by eluting them from the paper and then testing biologically. The rate of flow tested repeatedly in several different solvent systems chosen for their ability to separate catecholamines helps to identify the active substance. The rate of flow of known catecholamines are determined by adding standard solution to the paper. A method was described by Crawford and Outschoorn in 1951 (46) in which acid ethanol extracts were applied and chromatograms run by the capillary ascent method with phenol-water mixture with water saturated phenol applied in the bottom of the tank. A similar method has been described by James and Kilbey in

1950 (47). For larger amounts partition chromatography on a starch column with various solvent systems may be used with advantage. Ion exchange resin may be used for the separation of catecholamines in a mixture. Bergstrom and Hansson, 1951 (48) used amberlite and achieved a good separation of compounds. Lately, ion exchange resins, such as Dowex 50W received wide attention in the concentration, isolation and fractionation of active principles, hormones, amino acids, inorganic anions and cations from biological fluids and materials. The counter current distribution method of Craig (49), Bergstrom (50),and Euler (51) has been used for separation of catecholamines in a mixture of high purity such as after aluminium oxide adsorption.

d) Methods of Assay

After separation of the different catecholamines in an extract they may be assayed against a standard by any suitable biological or chemical method. Commonly used cytological method for the demonstration of catecholamines in certain tissues and organs has been to observe the chromaffinity of the cells. Recent work of Bertler et al. (52) indicate, that the chromaffinity of these cells is not due to adrenaline, noradrenaline or 5HT (5 hydroxytryptamine, serotonin) but to dopamine.

1. Colorimetric Methods

The reactions serving as a basis for colorimetric

methods can be brought about by various oxidants, such as iodine, ferricyanide, silver oxide and others. Upon oxidation both adrenaline and noradrenaline form colored quinone compounds. If the oxidation be made with iodine, the corresponding iodochrome is formed, which can be used for colorimetric determination. The inconvenience of the difference in color tints of the iodochromes of adrenaline and noradrenaline, when oxidized at different pH, can be avoided by use of an other oxidant such as permanganate (53). The method of Shaw is the most sensitive of all colorimetric methods (41). The method is based upon the observations of Whitehorn, 1935 - that adrenaline produces a blue color with arsenomolybdic acid by reduction. The method is used in combination of his adsorption method on aluminium hydroxyde. While colorimetric methods have led to substantial increases in knowledge they are much less sensitive and specific than the fluorometric methods.

2. Fluorometric Methods

Search for more sensitive and specific methods for catecholamines brought fluorescent procedures into use. In 1940 Hueber (54) reported making use of evanescent yellow-green fluorescence, which appeared when adrenaline solutions were make alkaline, for assay in the blood. Heller studied the procedure (55) and concluded that some oxidation product, related to adrenochrome was responsible for the

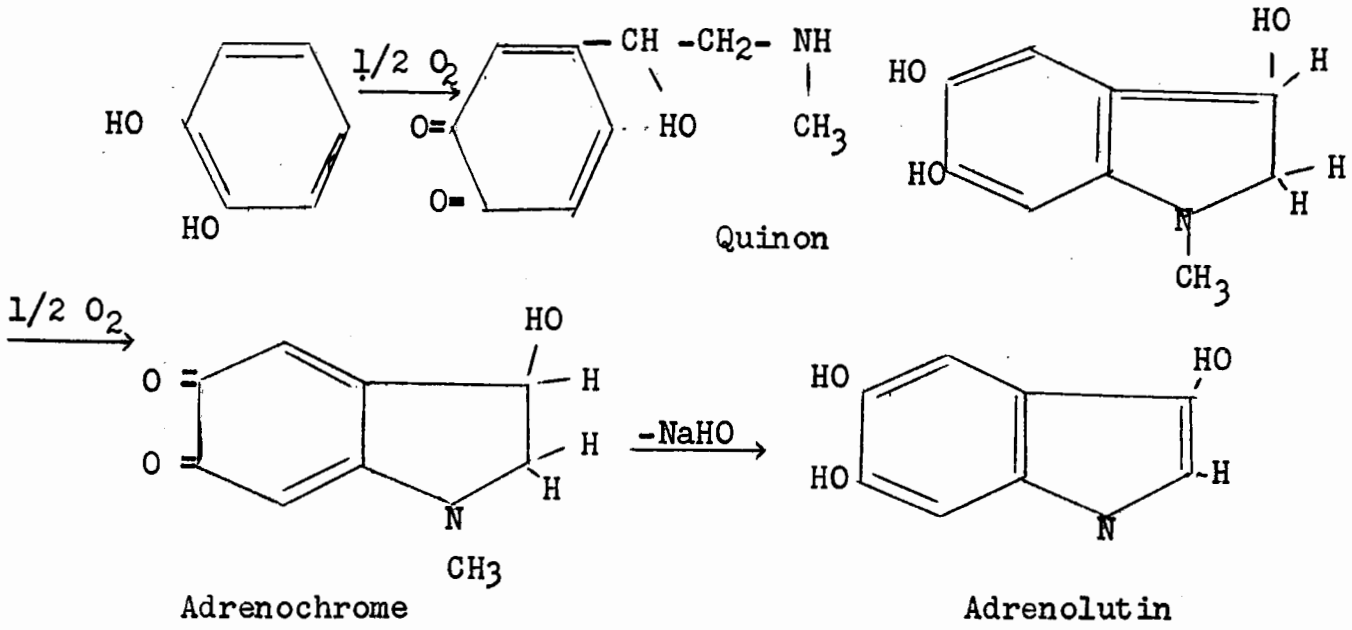
observed fluorescence. Studies by Lund (43) that the mechanism of oxidation and rearrangement of adrenaline in alkaline solution was elicited thus providing the information necessary to develop the trihydroxy indole procedure.

In order to emit fluorescent light a molecule must absorb light. However, not all substances which absorb light emit fluorescence, detectable with the available instruments. Thus benzene does not exhibit detectable fluorescence, but phenol, aniline, phenolic ethers, alkylated amines do. This led to the designation fluorophores. Polyphenols are also fluorophores, and since catechols are in this class, they fluoresce in their native form. Adrenaline, noradrenaline, dopamine are all activated at 285 μm and fluoresce at 325 μm, at pH 1. Because all the catecholamines have the same fluorescence characteristic, no distinction can be made.

Since the catecholamines are already fluorophores the necessity was for further chemical manipulation as the available instruments could activate only at certain wavelengths near the visible region, emitted light, fluorescence in the visible region, it was necessary to convert the molecule to some form which would meet these requirements (56).

The introduction of additional conjugated double bonds into the molecule, by shifting the absorption toward the visible region, also shifts the activation toward the visible. Thus by series of oxidations and rearrangements, the catecholamines are converted to polyhydroxyindoles, which are

fluorophores, absorb light near the visible range and emit visible fluorescence. The method of Euler and Floding is based upon these principles, where the catecholamines are adsorbed and eluted quantitatively on aluminium oxide before fluorescence procedure is brought about. The chemical sequence of events of trihydroxyindole procedure are as follows: (57)



Adrenochrome **Adrenolutin**

Another procedure for catecholamines involves condensation with reagents leading to visibly fluorescent polycyclic compounds. The observation by Natelson (57) that ethylendiamine condenses in such a manner with catecholamines was subsequently utilized by Weil-Malherbe and Bone (58). One of the most important factors to be considered is that all solutions, including the blanks, emit some light when activated. The light is composed of some true fluorescence and some scattered light (59). The limits of sensitivity of a given procedure are governed by this blank fluorescence and unless

the magnitude of this blank is known, it is impossible to evaluate the precision of a given procedure. The above two procedures have been widely utilized, both in their original form and with many modifications. It is rather difficult to determine which modifications are most suitable for a given problem, as to the best oxidant, the best pH values at which to carry out oxidations and the optimum means of measuring the resulting fluorescences.

Three characteristic reactions of adrenaline and noradrenaline have been used in their chemical estimation.

1. Chrome formation:- The catecholamines in the eluate are oxidized to form a mixture of coloured adrenochrome and noradrenochrome. Their concentration can be measured in a colorimeter at 529 μm, but the method has a limited usefulness (60).
2. Lutin formation:- Lutins are formed by alkaline rearrangement of the chromes of which they are actually isomers. They are unstable, but can be protected temporarily by ascorbic acid (61, 62). Oxidants convert adrenaline and noradrenaline to the corresponding lutins permitting measurement of their fluorescence.
3. Ethylendiamine condensation:- Under alkaline conditions catechol derivatives condense with ethylendiamine to form fluorescent substances (63). This is a very sensitive reaction and has been widely used for quantitative estimation of adrenaline and noradrenaline.

In order to determine adrenaline and noradrenaline in a mixture separately, two techniques and their combination have been used. The differences in the emission spectrum of the fluorescent products have been utilized by measuring fluorescence successively with two selected secondary filters (45). The second technique makes use of the greater rate of oxidation of adrenaline under selected reaction conditions, as at pH below 6, the so-called "differential pH method". In a fluorometric method for the determination of dopamine described by Carlsson and Waldeck in 1958 (64), the principal is similar to that employed in the trihydroxyindole method for estimating adrenaline and noradrenaline. Utilizing differences in fluorescence characteristic at pH about 5.3, microquantities of dopamine can be determined in the presence of at least equal amounts of adrenaline and noradrenaline.

Recently, a combined procedure has been described by Murphy and Sourkes (65) which permits the estimation of dopamine and noradrenaline independently of one another in a single sample of eluate. The trihydroxyindole method for adrenaline and noradrenaline and the Carlsson-Waldeck method (64) for dopamine were combined. Fluorescence characteristics of catecholamines obtained by these authors are shown on Table 1.

3. Bioassay Methods

The variation in activity ratio of various organs can be

TABLE I

FLUORESCENCE CHARACTERISTICS OF CATECHOLAMINES

Procedure			A	N	D
A[a]	Maxima[b]		410/520	390/505	410/510
	R.I.[c]	410/515	64	100	10
		365/485	3	58	0[d]
		365/510	23	77	4
B	Maxima		400/525		
	R.I.	400/525	100	0	0
		365/525	100	0	0
		365/485	100	0	0
C (acid)	Maxima				330/375
	R.I.	330/375	0	0	100
C (alkaline)	Maxima			400/520	
	R.I.	400/520	0	100	0
D (acid)	Maxima				330/375
	R.I.	330/375	0	0	100
D (alkaline)	Maxima		410/530	410/518	
	R.I.	410/515	20	100	5
		410/528	25	100	

a. Procedure A: Oxidation carried out using pH6 buffer.
" B: pH3 buffer used.
" C and D: see text.
b. Activation / fluorescence peak wave lengths (mμ).
c. Relative fluorescent intensities at indicated wave lengths.
d. Less than 1.0

utilized for the assay of adrenaline and noradrenaline in a mixture. However, the ratio is not constant under all conditions, it is connected with metabolic processes, endocrine activity, reproductive functions, seasonal variations, and foetal - adult life.

Description of Methods

1. Blood pressure:- Elliott (66) destroyed the spinal cord down to the 4th cervical segment, thus eliminating vasomotor reflexes. He injected a series of doses of synthetic adrenaline, turning the kymograph back so that the curves were superimposed. The drug was of constant volume, washed with constant volume of saline, the flow of injection checked with a metronome. The resulting curves from unknown solutions were correct slopes through their whole course because as he said: "the circulation then will respond with the accuracy of a chemical balance to any dose of adrenaline". The calculations were done by interpolation. Burn (67) using a similar technique, stated that the method is good to distinguish doses differing by 10%.
2. Rat uterus:- Jalon (68) utilized the abolition of spontaneous movement of the rat uterus suspended in an appropriate solution. Adrenaline was assayed by its effect in diminishing the response to a small dose of acetylcholine. Gaddum (69), Peart and Vogt (70, 71) suspended the uterus in a small bath. The content of the bath changed auto-

matically at regular intervals by solutions which ran in from below and overflowed. Carbachol 10^{-6} w/v applied for 30 sec. then washed out every 2 minutes. Thus regular contraction of the uterus was obtained. If adrenaline is applied 1 minute prior to the carbachol, the contraction is reduced and this reduction gives a measure of the dose of adrenaline. By this method 0.0001 µg adrenaline can be detected. In order to have effects like those of adrenaline, noradrenaline must be applied in amounts 100-200 times greater.

3. Blood-vessels of rabbit ear:- Schlossmann (72) perfused rabbit ears with a solution containing serum and citrate under constant pressure. The rate of flow of drops emerging is recorded after the solution to be tested is given. The method is sensitive to 10^{-11} w/v adrenaline. In a modified method of Savini (73), Page and Green (74) the interference with 5HT is excluded.
4. Intestinal muscle:- Methods were described by Stewart and Rogoff (75). They were sufficiently sensitive to be able to detect 10^{-9} w/v adrenaline.
5. Other tissues:- Isolated frog's heart - Loewi (13)
   Perfused frog's heart - West (76)
   Cat: nictitating membrane, uterus, heart - with intact circulation, Cannon (22).

As further preparation for bioassay all the tissues can be sensitized by: 1. section of the nerve supply,
2. injection of cocaine (71).

Tests for Specificity - Identification of Active Compounds: (77)

1. time-relationship of the effect
2. dose-effect curve difference
3. stability (such as rapid inactivation of adrenaline in alkaline solution)
4. antagonism; ergot alkaloids, phenoxybenzamine, etc.
5. chromatography
6. parallel pharmacological assays: Gaddum (78) and et al. (71)

If the unknown solution contains noradrenaline, it is almost always possible to find a dose of adrenaline which produces just the same effect in any single test, but if another test is used, the equivalent of adrenaline may be 10 or 100 times smaller. The value of any two tests for distinguishing between adrenaline and noradrenaline depends on the ratio of the results when these two substances are compared by the two tests. The ratio is called the index of discrimination.

By an appropriate choice of tests, it is possible to distinguish closely allied sympathomimetic amines from one another. The actual dose in these tests is usually not mentioned, the evidence depends entirely on a comparison of ratios. The method has been used by Cannon and Rosenblueth, von Euler and several others.

C. Occurrence of Catecholamines in Organs

a) Differences due to Methods

In the field of catecholamines, much of our present knowledge was attained by bioassay procedures. With the influx of biochemistry into the field of catecholamines, physical and chemical methods for estimating catecholamines became more and more popular. The subsequent development of more specific methods for the studies of small concentrations of catechol compounds has made it possible to obtain more satisfactory results. The technique of isolating them by using an adsorbent such as alumina was devised by Shaw in 1937. Following isolations many procedures have been utilized. Thus Loewi in 1937 (79) using chemical methods found evidence for the presence of adrenaline in extracts of frog and mammalian hearts. In extensive studies, Shaw 1938 and then Raab in 1943 found evidence for the presence of considerable quantities of catecholamines in the heart. Most of the studies were carried out by colorimetric technique at this time. The most popular method was based upon the blue color obtained with arsenomolybdate. The values reported by these methods were usually incredibly high by present day standards. Just a few years ago, papers were still appearing utilizing colorimetric procedures for estimating heart catecholamines.

Noradrenaline as a regular constituent of the heart was first detected by von Euler in 1946 (80). The ratio of adrenaline to noradrenaline in the heart was studied by Goodall 1951 (30) and by Holtz 1951 (81). They both found that the adrenaline content was relatively high in comparison to other organs.

The fluorometric technique for the estimation of the catecholamines has been used by many authors since the fluorescence of adrenaline in an alkaline solution was discovered by Paget in 1930. A strong fluorescence due to adrenaline was demonstrated in extracts of frogs' hearts by Loewi in 1936. In recent years, the most reliable data presented were estimated by fluorometric techniques.

b) Species Differences

The first indication of the presence of a sympathomimetic substance in adrenergic nerves was obtained by Gaddum and Khayyal in 1935 (82). The first extraction of adrenergic nerves seem to have been made by Lissak in 1939 (83), who demonstrated that such extracts had adrenaline-like actions. Further analysis by von Euler in 1946-1948 showed that the activity of the nerve extracts was chiefly due to noradrenaline, though minute quantities of adrenaline were also present. It could not be ascertained whether or not these small amounts originated from the adrenergic nerves proper.

A systematic study of extracts of various nerves from mammals, including man, and non-mammalian animals, showed that the amount of catecholamines varied considerably in nerves of different species. The relative content of noradrenaline in different organs and tissues bears an obvious correlation to their adrenergic nerve supply and function.

Tables II, III, IV and V show the noradrenaline, adrenaline and dopamine content of different organs in mammalian and non-mammalian animals.

Significant amounts of catecholamines have been found in fruits, like the banana (84) and in certain insects (85). The significance of their presence has not yet been established.

c) Comparison of the Catecholamine Content of Various Organs

In 1948, Holtz (86) presented pharmacological evidence that beef adrenal glands contain noradrenaline in addition to adrenaline. The absolute as well as the relative figures for noradrenaline in the gland vary greatly. There are certain indications of grouping of the different animals with regard to the relative noradrenaline content of the adrenals. Goodall (87) postulated that a high amount of noradrenaline occurred in aggressive animals in contradistinction to the low figures found in non-aggressive species. Hökfelt (88) reported findings indicating that the relative noradrenaline content in one and the same species was remarkably constant and therefore typical. It was noticed by Hokfelt in 1951 (89) that the relative amount of noradrenaline was very much higher in fetal adrenals than in adults. - Table VI. The mechanism and functional significance of the low absolute and relative adrenaline content of the gland has not been elucidated.

TABLE II

Noradrenaline µG./G. In Organs

Organ	Horse	Cow	Sheep	Pig	Dog	Cat	Rabbit	Guinea Pig	Rat
Spleen	3.0 2.7(B&F)	1.50-3.50 4.70(B&F)	1.60-3.30		0.4-1.0 0.2(B&F)	0.80-1.40	0.3-0 5	1.5	0.40 (E&H)
Heart		0.48(H-t) 0.30-0.60(G)	0.80(G) 0.60-1.10	0.3-0.50 (H.K.&S)		0.50-1.00	0.5(H-t) 0.5	0.4-1.0 (H.K.&S)	0.65(H-t)
Salivary glands			0.40-2.20			1.40			
Lymph		0.50-0.80	0.30						
Kidney		0.04-0.10	0.40-0.60			0.10-0.30			
Ciliary body and iris		0.40							
Arteries	0.5-2.0(S)	0.20-1.00(S)		1.50(S)	1.5(S)				
Veins		0.10-0.50							
Liver		0.25 0.20(H-t)	0.20-0.50			0.05-0.20			0.06(H-t)
Lung		0.05	0.10						
Intestine		0.15							
Uterus		0.15							
Intestine			0.10						
Testicle		0.04							
Skeletal muscle		0.04	0.03-0.07			0.03			
Bone marrow		0.00							
Placenta									0.00

B&F - Bacq and Fischer, E&H - Euler and Hökfelt, H-t - Hökfelt, G - Goodall, H.K.& S - Holtz, Kroneberg & Schümann, S - Schmiterlow

Euler U.S. v.: Noradrenaline Charles C. Thomas, Springfield, Ma.

TABLE III

Noradrenaline in Organs of Poikilothermic Animals

Animal	Organ	Noradr. µg./g.	Adr. µg./g.	% Adr.	Author
Frog	Heart			100%	Euler (1946c)
(Rana temp.)	Heart	0.10-0.340	0.76-1.000	75-91%	Östlund (1954)
(Rana temp.)	Spleen	0.52;0.750	0.21;0.380	22;42%	Östlund (1954)
Toad					
(Bufo gargarizans)	Venom			96-98%	Lee & Chen (1951)
(Bufo agua)	Venom			95-98%	Lasagna (1951)
(Bufo arenarum Hensel)	Venom				Cabib (1951)
Dogfish (Squalus)	"Ganglion chain"			29%	Euler (1953)
Salmon	Heart	0.029	0.042	59%	Euler (1953)
Salmon	Liver	0.020	0.020	50%	Euler (1953)
Salmon	Spleen	0.029	0.042	59%	Euler (1953)
Octopus vulg.	Post. salivary glands	1.00-3.000			Euler (1953)
Lumbricus	Ganglion chains	0.320	1.400	81%	Östlund (1954)
Tenebrio molitor	Whole body larvae	2.200	0.061	3%	Östlund (1954)
Tenebrio molitor	imago	1.100	0.150	12%	Östlund (1954)
Apis mellifica (worker)	immature pupa	0.050	0.050	52%	Östlund (1954)
	larvae	0.300	0.005	2%	
Apis mellifica (worker)	imago	0.750	0.050	5%	Östlund (1954)

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TABLE IV

Noradrenaline and Adrenaline Content in Various Organs of the Sheep

Organ (Sheep)	Noradrenaline	Adrenaline	Adrenaline %
Spleen	3.0 - 3.3	0 -0.11	0.- 3.4
Parotid Gland	0.5 - 2.2	0.03 -0.19	5 - 14
Submaxillary Gland	0.4 - 1.1	0.10 -0.21	12 - 21
Heart	0.6 - 1.1	0.1 -0.2	10 - 20
Kidney	0.4 - 0.6	0.05 -0.07	11
Liver	0.15- 0.20	0.007-0.011	4 - 7
Lung	0.08- 0.1	0.002-0.01	2.5 - 10
Striated Muscle	0.025	0.0013	5
Brain	0.08		
Sciatic Nerve	0.14		
Mesenteric Nerves	3.5		
Splenic Nerves	8.0		

U.S. v. Euler Epinephrine and Norepinephrine

Pharmac Rev. 6: 17, 1954.

TABLE V

Noradrenaline and Dopamine in Mammalian Organs in µg/g.

	Heart		Lungs		Spleen		Liver		Kidney		Duod.		Sympath. Trunk		Sciatic	
	NA	DA	NA	DA	NA	DA	NA	DA	NA	DA	NA	DA	NA	DA	NA	DA
Cow	1.0	1.5	0.1	0.5	1.0	0.8	0.1	2.5	0.2	0.2	-	-	-	-	-	-
Sheep	1.0	0.3	0.1	6.9	1.9	0.9	0.2	0.3	0.0	0.0	-	-	-	-	-	-
Goat	1.4	0.5	0.1	5.3	6.8	1.0	0.1	1.6	0.7	2.0	0.2	6.3	1.2	0.6	0.1	0.4
Pig	-	-	0.1	0.0	-	-	-	-	-	-	-	-	-	-	-	-
Dog	1.0	0.0	0.1	0.0	1.1	0.0	0.3	0.0	1.3	0.0	-	-	1.8	0.1	-	-
Cat	1.2	0.0	0.3	0.0	2.9	0.1	0.1	0.0	0.1	0.0	0.5	0.2	-	-	-	-
Rabbit	1.4	0.0	0.0	0.2	-	-	0.1	0.0	0.2	0.0	0.4	0.2	-	-	-	-
Guinea pig	2.6	0.0	0.2	-	-	0.4	0.2	0.0	0.6	0.0	-	-	-	-	-	-
Rat	0.8	0.0	0.1	0.0	0.0	0.6	0.1	0.1	0.0	0.1	0.0	-	-	-	-	-

Carlsson, A.

Symposium on Catecholamines 1958, The Williams and Wilkins Co. Baltimore, Maryland.

TABLE VI

Catecholamine Content of the Adrenals Glands of Various Species

Species	Age	No. of Observations.	Noradrenaline: µg./pair of Adrenals Range	Adrenaline: µg./pair of Adrenals Range	Adrenaline in Percentage of Total Catechols; Mean
Rat	4 days before birth	2	0.000	0.000	0
Rat	3 days before birth	1	0.012	0.003	20
Rat	2 days before birth	4	0.013-0.018	0.007-0.009	34
Rat	1 day before birth	2	0.019-0.020	0.015-0.016	43
Rat	2 days after birth	4	0.310-0.390	0.400-0.510	56
Rat	6 days after birth	3	0.631-0.681	0.920-1.120	60
Rat	13 days after birth	5	1.090-1.240	1.990-2.130	64
Rat	40 days after birth	2	2,590-2.900	14.80-15.30	85
Rat	120 days after birth	3	7.420-7.780	38.10-42.00	84
Guinea pig	At birth	3	3.750-4.050	15.50-17.00	81
Guinea pig	Adult	8	0.000	64.60-74.10	100
Rabbit	At birth	3	2.320-2.940	3.980-4.520	62
Rabbit	Adult	8	0.000	78.20-89.40	100
Cat	2 weeks before birth	1	2.400	0.000	0
Cat	At birth	3	27.20-29.30	13.80-17.20	36
Cat	Adult	4	130.0-138.0	131.0-138.0	50
Man	28 weeks before birth	4	0.720-1.200	0.000	0
Man	22 weeks before birth	6	24.00-26.40	2.200-3.400	10
Man	18 weeks before birth	3	26.90-32.10	4.080-5.100	14
Man	At birth	5	36.80-51.00	15.50-22.80	30
Man	2 years of age	2	44.50-46.50	61.50-69.50	59
Man	45 years of age	3	172.0-196.0	922.0-988.0	84
Man	60 years of age	4	241.0-298.0	1554.0-1680.0	88
Man	75 years of age	5	149.0-197.0	2154.0-2354.0	93

Hökfelt, B. Acth physiol. scandinav:25 : Suppl. 92, 1951 A.

Tissues which accumulate catecholamines have become known as chromaffine tissues because of their ability to show a characteristic histological staining reaction due to reduction of chromates. In certain instances apart from the adrenal gland, paraganglia, Zuckerkandl-body, the presence of adrenaline-like activity in organs has been accounted for by the demonstration of chromaffine cells in such organs. A study of a great variety of organs and tissues has revealed that practically all of them contain noradrenaline, some of them contain adrenaline or dopamine, though the content varies considerably as seen on Tables II, III and IV.

The heart contains somewhat less than the spleen, the liver still less and the skeletal muscle very small amounts of noradrenaline. Noradrenaline could not be demonstrated in the placenta (90) which is in agreement with the fact that this organ lacks nerves. The noradrenaline content of the lung is quite low, but a large amount of dopamine has been found (91). The brain in addition to noradrenaline and dopamine contains a certain amount of dopa, too. Whether the brain contains adrenaline or not, is still disputed. Detailed monographs are available (92, 93) on the relative concentration of catecholamines in different parts of the brain (Tables VII and VIII). Two puzzling facts still await interpretation. Firstly, the occurrence of large amounts of enzyme (L-dopa decarboxylase) in organs like the kidney and

TABLE VII

Occurrence of adrenaline, noradrenaline, and dopamine in the brains of different species of animals

	Adrenaline	Noradrenaline	Dopamine
	µg/g	µg/g	µg/g
Sheep	-	0.25	0.30
Pig	-	0.14	0.22
Dog	-	0.16	0.19
Cat	-	0.16	0.22
Rabbit	-	0.22	0.28
Guinea pig	-	0.38	0.34
Rat	-	0.49	0.60
Frog and toad	1.4	0.26	-

Carlsson, A - Pharmac. Rev. Vol. 11, No. 2, Part II, 1959

TABLE VIII

Distribution of noradrenaline and dopamine in the dog brain

	Noradrenaline	Dopamine
	µg/g	µg/g
Cerebral hemispheres (not corpus striatum, hippocampus)		
rostral part	0.13	0.07
caudal part	0.12	0.08
Caudate nucleus	0.10	5.90
Lentiform nucleus	0.08	1.63
Hippocampus	0.14	0.13
Hypothalamus	0.76	0.26
Diencephalon (not hypothalamus)	0.17	0.09
Mesencephalon	0.33	0.20
Pons	0.41	0.10
Medulla oblongata	0.37	0.13
Cerebellum	0.06	0.03

Carlsson, A- Pharmac. Rev. Vol. 11, No, Part II, 1959

the liver (94, 95), locations for which a high turnover of catecholamines has never been demonstrated, and secondly, the high dopamine content of the lung from which L-dopa decarboxylase is absent (96). There exists a possibility that the precursors of adrenaline might reach other tissues by way of the blood stream, or that dopamine itself is the end product of biosynthesis in these organs (97).

d) Catecholamines with Special Reference to the Heart

Muscholl (98) extracted pieces of heart tissue of cats, rabbits, guinea-pigs and rats and bioassayed them for catecholamines. In all species, the noradrenaline concentration was much higher in the right than in the left atrium, and higher in the right than in the left ventricle. Approximately 4% of the total amines were adrenaline. He suggested that the small percentage of the adrenaline indicates that the heart of these species contain little chromaffine tissue. The high concentration of noradrenaline in the right side of the heart was supposed to be due mainly to the presence of adrenergic fibers. Raab reported (99, 100) that the heart of the above mentioned species contains invariably 20% of adrenaline, in contrast to the other organs where a much lesser percentage is found.

Muscholl (101) suggested that the adrenaline of the heart behaved differently from that of the sympathetic ganglia or postganglionic nerve fibers after an injection of reserpine. In the heart, the concentration of noradrenaline

was reduced by 93-97% and the adrenaline by 59-79%, while in the ganglia or in the nerve fibers, the reserpine did not reduce the concentration of adrenaline. Bertler et al. (102) indicated that the dopamine content of the ruminants' tissues varies with the numbers of a special type of chromaffine cells and that there is a close relation between the distribution of the dopamine and these cells within certain organs. In the cow heart, few chromaffine cells were seen in the endocardium, pericardium and in the interstitial tissue of musculature. This hardly seems to agree with the high dopamine content. A similar discrepancy was seen in the spleen of these animals.

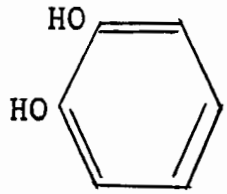
Goodall and Kirschner (103) subjected sheep and dogs to various stages of cardiac sympatho-gangliectomy, i.e. right cervical, right thoracic, left cervical and thoracic. In sheep, the right cervical and the right thoracic cardiac ganglia were the cardiac ganglia that influenced to the greatest extent the noradrenaline content of the sheep heart. Upon removal of either of these ganglia groups the cardiac noradrenaline was reduced to approximately one-half and if both were removed the noradrenaline titer was reduced to about one-sixth of the normal level. In dogs, the right cervical cardiac ganglia significantly influenced the noradrenaline content of the heart. Upon removal of the right inferior and middle cervical ganglia, the cardiac noradrenaline was reduced to approximately one-third of its

normal level. In both dogs and sheep, removal of the cardiac sympathetics had no consistent effect upon the adrenaline content of the heart. Regeneration in terms of noradrenaline content required 5 - 16 weeks.

Esko and Pekkarinen (104) found that amine oxidase and cytochrome oxidase are active in heart muscle in vitro. Axelrod (105) gave evidence for the occurrence of the catechol-O-transferase in the heart.

Schmitterlow (106) gave evidence that the coronary vessels were particularly rich in noradrenaline and Outschoorn and Vogt (107) demonstrated that upon sympathetic stimulation there was a release of the noradrenaline only to the coronary circulation. Electrocardiographic changes induced by adrenaline and noradrenaline have been described by numerous authors. The therapeutic implications of the differences in action on the heart between noradrenaline and adrenaline have also been discussed by numerous authors.

D. Biosynthesis of Catecholamines



Catechol (1,2 Dihydroxybenzene)

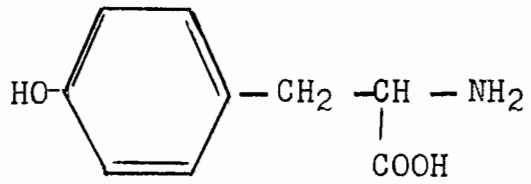
The chemical constitution of adrenaline and other catecholamines have been known for over half a century, but it is only now, that we can talk with some degree of confidence about the intermediate stages of catecholamine formation in

the animal body. The steps that occur in pathways of the formation of tyrosine metabolism and the formation of noradrenaline and adrenaline were first postulated as early as 1939 by Blaschko (108). The advent of radioisotope techniques made it possible to confirm his views convincingly.

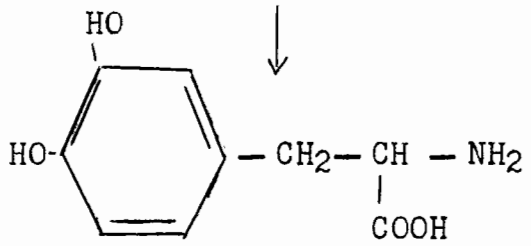
Early attempts to demonstrate the formation of adrenaline produced conflicting and equivocal results mainly because of difficulty in detecting small amounts of newly formed adrenaline and noradrenaline in the presence of large amounts of preformed hormones. The problem was greatly simplified by the introduction of radioactive tracers and techniques of ion exchange and filter paper chromatography. Kirschner and Goodall (109) incubated uniformly labeled l-tyrosine C^{14} with slices of adrenal medulla, and dopamine C^{14}, noradrenaline C^{14}, and adrenaline C^{14} were isolated by exchange chromatography. Under identical conditions, tyramine C^{14} did not serve as a precursor of noradrenaline. Similar results were obtained by isotope dilution experiments (110). Incubating sympathetic nerve tissue and ganglia, the amount of radioactivity in the adrenaline fraction was quite low and could have been due to contamination.

Table IX shows that in the formation of catecholamines the first step involves dietary phenylalanine which by enzymatic catalysis, by folic acid, is transformed to tyrosine. From tyrosine, the path is by a non-enzymatic route (Fe^{+++}, ascorbic acid) to dopa (111, 112, 113). The role played by

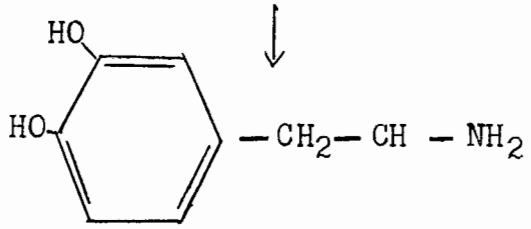
TABLE X



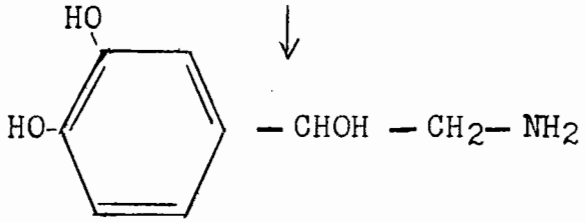
Tyrosine



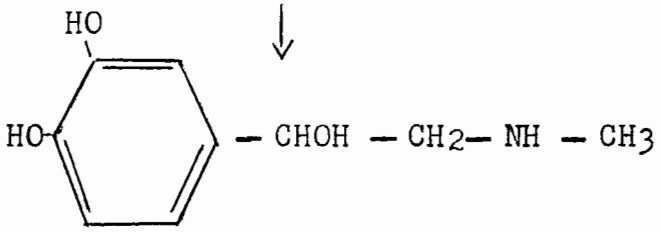
Dopa



Dopamine



Noradrenaline



Adrenaline

Main pathway of formation of catecholamines

l-dopa decarboxylase in the biosynthesis of catecholamines depends on the organ in which the primary product of its action, dopamine is formed. The enzyme was discovered in 1938 by Holtz (114) and Blaschko (108). In biological tests, dopamine was found to be 50 - 100 less active than the adrenaline. For that reason, we believe that the physiological role of l-dopa decarboxylase was not so much to form a pharmacologically active amine from an inert amino acid but rather to form the precursor for the biosynthesis of adrenaline. The enzyme strongly requires pyridoxal-5 phosphate as a coenzyme for its action (115). There exists an appropriate correlation between the l-dopa decarboxylase activity and the noradrenaline content of the sympathetic nerve tissues and brain (116). However, since large amounts of enzyme are found in organs like the liver and kidney when a high turnover of catecholamines has never been demonstrated, the possibility is raised that the precursors of adrenaline might reach other tissues by way of the blood stream. Possibly in these organs, dopamine itself is the end product of biosynthesis and acts as local hormone. The high dopamine content of the lung from which the enzyme is absent and that of the brain and nervous tissue contain nearly as much dopamine as noradrenaline also supports this previous suggestion. Dopamine is quickly formed by decarboxylation and noradrenaline probably formed slowly from dopamine. There is no clear-cut evidence

that nervous tissue is able to carry out methylation to adrenaline in contrast to the chromaffine cells of the adrenal medulla (111). According to the accepted pathway, the immediate precursor of adrenaline is noradrenaline. Noradrenaline has a dual role, therefore, not only as a neuro-hormone, but also as an intermediate in adrenaline formation.

In recent years, we have learned more about the biochemistry of methylation of noradrenaline to adrenaline and about the part played by S-adenosylmethionine as a methyl-donor in this reaction. Cantoni (117) demonstrated the supernatant fraction of beef adrenal medullary homogenates to form adrenaline C^{14} from noradrenaline, ATP and methionine-methyl C^{14}. The first step involves the reaction between ATP and methionine to form S-adenosylmethionine, the second, the transfer of a methyl group to the amino group of noradrenaline. When methionine is used as a methyl-donor, ATP and Mg^{++} are absolute requirements, when S-adenosylmethionine is the methyl-donor Mg^{++} and glutathione stimulate but are not required.

No convincing evidence exists for the possible existence of secondary pathways in catecholamine formation such as hydroxylation in the side-chain with the formation of dihydroxyphenylserine preceding decarboxylation (112). In octopodes, there is an absence of the reaction in which the second phenolic hydroxyl group is introduced (118, 119).

E. Storage and Release of Catecholamines

Available evidence suggests that catecholamines are present in nervous tissue in a form which can be readily mobilized. Electrical stimuli of such nerves lead to the release of sympathomimetic amines (120). The adrenaline and noradrenaline producing systems can be activated separately and independently of each other, indicating a duality of function. It was established (121) that the resting secretion of adrenal medulla is lo^{-9} w/v which is well below the range of observable effect. Noradrenaline and dopamine are present in nerve trunks and there are reasons to believe that the amine content is greatly increased in the region of nerve endings (122). Luco and Goni (123) demonstrated that the catecholamine content of sympathetic nerves and organ content are no different after electrical stimulation. The catecholamines in the nerve trunk are never released under physiological conditions. On the other hand, the lack of reduction might be due to rapid resynthesis which probably occurs in a terminal part. This consideration implies that the heart and other organs contain catecholamines in stored, inactive form. Activation of these stores is accomplished by sympathetic discharge or by other means. Under these circumstances, the catecholamines are transformed from the stores to an active form.

Adrenal medullary cells are ontogenically sympathetic nerve cells, modified so that they liberate their secretion directly at the surface of the cell body and

thence into the circulation. The catecholamines are not evenly distributed throughout the cells but are held in specific cell organelles, like other biologically active amines. In electronmicrographs, these chromaffine granules are circular, their diameter is usually between 50 - 90 μm. They are smaller than the mitochondria and appear to be distributed in the cytoplasm in smaller or larger groups (124). The granules are the most concentrated source of ATP, where the molar ratio of amines to ATP is 4:1 (125). It seems that ATP serves as an anion paired with the basic catecholamines. Possibly in the storage position, one of the four negative charges of ATP molecule is not used to neutralize a positive charge of catecholamines, but it may be used for anchoring the ATP in the storage position to a receptor protein (126). The methylation of noradrenaline takes place outside of the granules. The hydroxylation of dopamine to noradrenaline is a function of the unseparated granule fraction, but whether the chromaffine granules or mitochondria is responsible remains undetermined (127). Dopamine in the adrenal medulla is intragranular, but in the sympathetic nerves, is found principally in the non-particulate cytoplasm (128). The termination of action of catecholamines is brought about by several different means.

F. Metabolism of Catecholamines

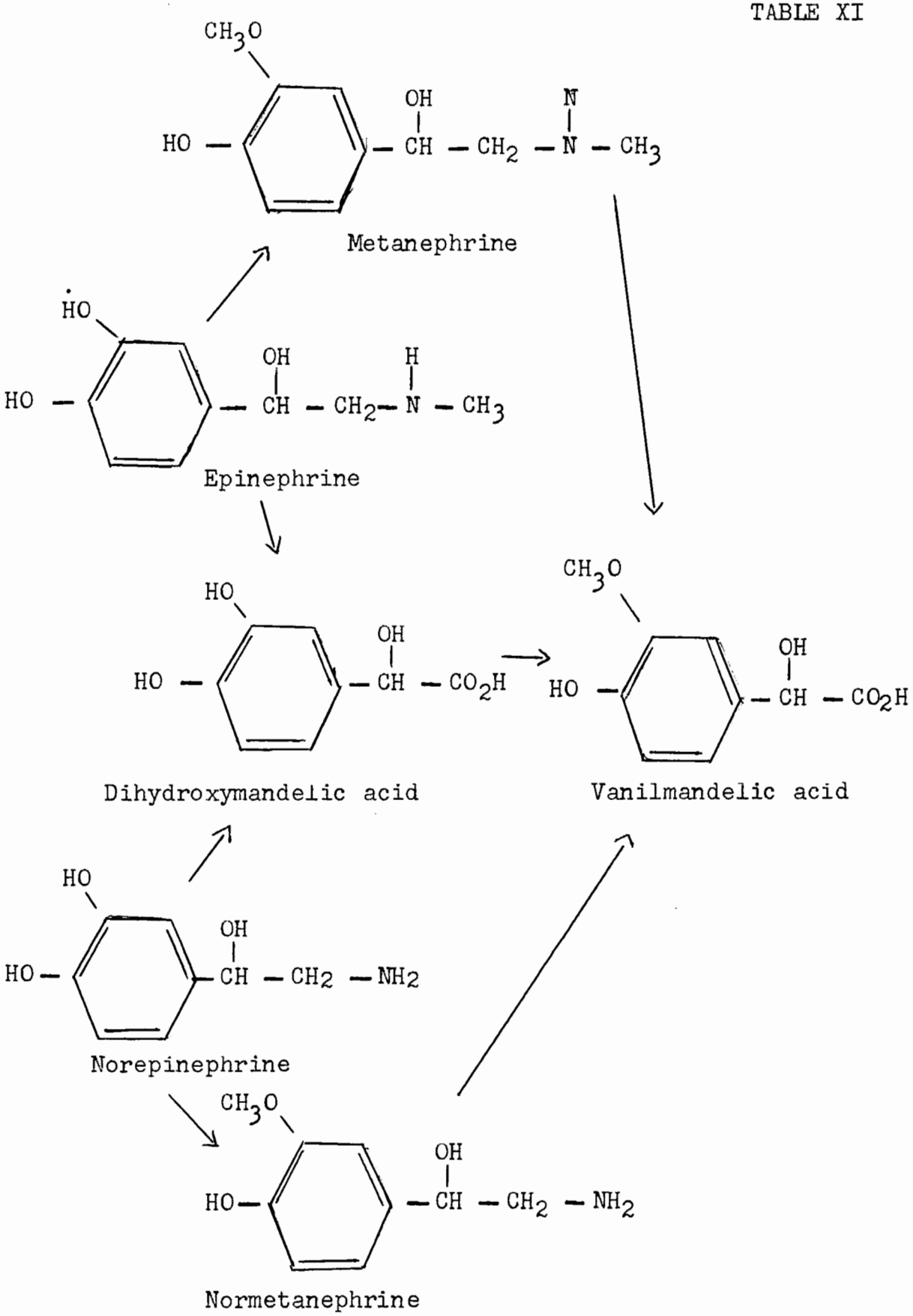
In 1937, Green and Richter reported that cytochrome

oxidase can convert adrenaline to adrenochrome (129). However, Schayer (130) and Szara (131) using sensitive radioactive methods failed to substantiate this, and we can assume that endogenous or administered adrenaline can be accounted for by a number of metabolites, none of which have been identified as adrenochrome. According to recent works, it is now generally believed that conjugation plays a minor role in inactivation (132).

The introduction of potent MAO inhibitors, iproniazid and choline-p-tolyl ether made possible the elucidation to a certain extent the role of MAO in the metabolism of catecholamines. Whether deamination occurs on the adrenaline molecule itself or an amine containing metabolite is not clearly established. There are suggestions that MAO more readily attacks the O-methylated metabolites (132). Recently, Armstrong and co-workers have shown (133) that a major metabolic product of noradrenaline in man is 3-methoxy4-hydroxy mandelic acid. In Axelrod's laboratory, the O-methylation of administered catecholamines was demonstrated as well as the normal occurrence of metanephrine (3-O-methylepinephrine) and normetanephrine (3-O-methylnorepinephrine) in urine and certain tissues (134). In addition, the enzyme, catechol-O-methyltransferase, has been found (135). In light of these findings, it can be concluded that the principal pathway for the metabolism of adrenaline and noradrenaline in man and rodents is the O-methylation to metanephrine or normetanephrine,

METABOLISM OF EPINEPHRINE AND NOREPINEPHRINE

TABLE XI



in turn deaminated by MAO or conjugated. Direct deamination of the catecholamines appears to be a minor route of transformation.

G. Physiology of Catecholamines

a) Redistribution

Raab and Gigee reported (136) that adrenaline and noradrenaline were selectively taken up by heart muscle and other vascular tissues such as the spleen, when massive doses (2 mg/kg) of these compounds were administered to cats or dogs. More recently, Axelrod et al. (137) examined the physiological disposition of adrenaline and its metabolite metanephrine in cats and mice after an i.v. infusion of a physiological amount of tritium labeled -DL-H^3- epinephrine of high specific activity. The amines were separately determined by specific procedures involving column chromatography and extractions in organic solvents. Immediately after the end of a 30 min. infusion (3 g/min/kg) the concentration of the labeled epinephrine in the heart, spleen, adrenal and pituitary gland exceeded that of plasma several fold. In the kidney, liver, lung and intestines, its concentration was of the same magnitude as that of plasma, while it was lower in skeletal muscle. Two hours after the administration of labeled epinephrine, large quantities were found in the heart and spleen indicating that these tissues not only accumulate but also retain it for long periods of time.

The concentration of metanephrine in plasma immediately after the infusion was about the same as that of epinephrine. The O-methylated metabolites accumulated in the heart, spleen, adrenal gland, liver but an insignificant amount was found in the brain.

b) Tolerance, Tachyphylaxis and Related Phenomena

It has been known for a long time that a large number of sympathetically innervated structures, including the heart, if deprived of their pre and postganglionic nerve supply become hypersensitive to administered catecholamines (138). According to von Euler, degeneration of the autonomic nerves by section of the postganglionic fibers did not abolish the effect of catecholamines on the denervated structure (139). Hence, the receptive substance is part of the cell and trophically dependent on it. There is no evidence that catecholamines require the presence of nerve endings for their action. Catecholamines also act on naturally nerve-free smooth muscles such as the placental vessels.

c) Vasosensory Mechanism

Some of the differences observed between the action of noradrenaline and adrenaline on the heart do not appear when the organ is isolated. Bradycardia following noradrenaline administration is abolished by atropine (140). Gollwitzer and Meier reported (141) that bradycardia can be elicited from receptors in the heart itself. Specific

mechanism of the autonomic regulation, such as the carotid sinus and the cardio-aortic vasosensory mechanisms play a significant role in the functional balance. Low blood pressure, low oxygen tension or excessive carbon dioxide in the carotid sinus tend to inactivate the carotid sinus mechanism, reduce its inhibitory action, release the restrained sympathetic mechanism from tonic inhibition. Catecholamines in the circulating blood tend to sensitize the carotid sinus mechanism and thus increase its inhibitory effects, both on blood pressure and respiration. It also increases the sensitivity of the carotid sinus mechanism to the existing blood pressure, consequently, a depressor reaction may be produced independently of the peripheral depressor effect of catecholamines. Catecholamines also exercise an inhibitory control over adrenal medullary secretion and thereby provide homoestatic limitation on its own account (142).

d) Receptors for Catecholamines

The manner in which catecholamines make contact with effector cells is important to an understanding of the function of the mechanism of such a highly organized regulatory system. Receptors are sites of the action of catecholamines. The original classification of Dale (144) based on ergot. alkaloid paralyzing action, recently has been greatly modified. We now differentiate receptors as

those:

1) on the surface membrane
2) intracellular receptors, probably acting on enzyme systems (143).

Ahlquist (145) classified the receptors mediating specific responses in different effector organs largely on the basis of order of potency of a series of five sympathomimetic amines eliciting these responses. He spoke of alpha and beta receptors, where the latter could not be blocked by blocking agents. He indicated that in the heart, there were only beta receptors on which the amines effecting an increase in rate and strength of heart-beat operate. Lands (146) objected to this grouping and preferred to classify adrenergic receptors in the heart as undifferentiated (Acr), since this organ is stimulated by substances with strong affinity for either the excitatory receptors (Ac) or the inhibitory receptors of the smooth muscle (Ar). His work was based upon a study of the characteristics of the molecule of the sympathomimetic amines, which determine the effect with regard to excitatory or inhibitory action.

e) Adrenergic Blocking Agents

None of the wide variety of natural and synthetic agents which block the excitatory effects of adrenaline in smooth muscle is capable of giving clear-cut blockade of its excitatory effects in mammalian heart. Generally, adrenergic blocking agents do not alter the chrono and inotropic effect of adrenaline on the heart, but effectively prevents the

serious arrhythmias caused by the drug. Recently, a new agent 1 (3' - 4' dichlorophenyl) 2 isopropyl aminoethanol (the dichloroanaloge of isoproterenol) has been claimed to be potent in blocking ino and chronotropic action of catecholamines on the heart (143). There were, however, doubts raised that the agent has no direct action, but suppresses the myocardium and possibly acts on cardiac glycosides or calcium.

f) Physiological Action of Catecholamines

The actions of noradrenaline on the heart is in many respects identical with those produced by stimulation of the cardiac sympathetic nerves. This is in agreement with the fact that the chief sympathomimetic substance of the heart is noradrenaline (142). Heart extracts in addition contain a certain amount of adrenaline, which may be as high as 20% of the total catecholamines. On stimulation of the accelerator nerves in the dog, Outschoorn and Vogt found (107) that only noradrenaline was released into the coronary blood. The action of noradrenaline and adrenaline on the coronary vessels and circulation has been the subject of controversial opinions not so much with regard to the actual effect, but rather with respect to the mechanism. During normal conditions, it is usually not possible to decide whether a dilator action on the coronary circulation is secondary to increased cardiac activity. In spite of myocardial stimulation, Lu

and Melville (147) found a decreased coronary flow per heart beat with noradrenaline, except in large doses. In several experiments, it was possible to demonstrate a pure dilator action with both adrenaline and noradrenaline (148) however, a constrictor action of adrenaline on the coronary vessels has been described on the basis of histological observations by Hirsch (149) in 1952. A comparison of the action of adrenaline and noradrenaline seems to indicate that while both substances dilate the coronary vessels in the dog, that adrenaline was stronger (150).

The released and injected catecholamines have quantitatively and qualitatively the same physiological action and in the case of the heart, non-physiological effects. According to Koelle, these are (151):

1. Excitatory action on autonomic effector cells.
2. Inhibitory action on autonomic effector cells.
3. Metabolic action.
4. Action on CNS.

According to Nickerson (152) the cardiac effects of catecholamines can be further subdivided into:

a. physiological
b. non-physiological and pathological responses, the production of arrhythmias on the basis of resistance or susceptibility to inhibition by common adrenergic blocking agents.

By injecting 0.7 mg Epinephrine Hydrochloride s.c. in a

human being under basal conditions, the cardiovascular effects are as follows, according to Starr et al. (153):

	% decrease	% increase
Pulse rate		21.0
Systolic pressure		4.8
Diastolic pressure	14.5	
Cardiac output		51.7
Stroke volume		22.7
Left ventricular work/min		52.4
Left ventricular work/beat		26.2
Heart volume	4.0	
Metabolic rate		30.4
A-V O_2 difference	13.3	

After administration of the drug, it can be readily seen that it stimulates the heart by direct action on the myocardium, independently of alterations in cardiac functions which are secondary to the effects on peripheral circulation. The rate is accelerated (positive chronotropic effect), and the rhythm is often altered. Cardiac systole is often shortened but more forceful and cardiac output is enhanced (positive inotropic effect). The work of the heart and the oxygen consumption of the myocardium are markedly increased. The drug tends to shorten the refractory period of the atrial muscle, speeds atrio-ventricular conduction, decreases the grade of A-V block. Premature systoles of ventricular origin occur. When other factors affecting cardiac irritability are

also present, it is likely to precipitate extrasystoles, tachycardia and fibrillation of ventricular origin (154).

Mention has been made of the dual function of adrenaline and noradrenaline producing systems. The principle organ for adrenaline is the adrenal medulla, chromaffine tissue, remnants of embryonic paraganglia and in certain species, the carotid body (155). It is well accepted now that the transmitter substance of sympathetic nerves is noradrenaline. The resting secretion of adrenaline is below effective range (121) and the firing at the nerve ending is varied to bring about the moment to moment adjustment of homeostasis. The primary action of adrenaline appears to be on the metabolism, which possibly and indirectly potentiates the effect of noradrenaline. Nickerson and Nomaguchi (156) working with the frog heart showed that the adrenaline induced increase in heart rate is dependent on:

1. stimulation of the production of the utilizable acetate,
2. a trigger action effective only in the presence of suitable acetyl substrate. They speculated that adrenaline promoted the metabolic substrate required for chronotropic action.

Shanes (157, 158) proposed that accumulation of organic phosphate as a product of glycogenolysis was responsible for changes in ionical and electrical characteristics. These acids with indiffusable anions might

reduce potassium and phosphate linkage and also contribute hydrogen ions to augment the potassium ion uptake by exchange through the membrane. Such ionic interaction leads to a better sustained membrane potential and hyperpolarization.

Eccles finding (159) that inhibitory impulses produces hyperpolarization was new and unexpected. He accomplished a great deal in the elucidation of inhibition by microelectrode impalement of motor neurons of the spinal cord of the cat. He was able to record the resting and action potential of the membrane of a single cell and determine the effects of excitatory and inhibitory impulses on the synaptic and spike potentials of the neuron. De and repolarization of a nerve or muscle are the result of movement of sodium into and out of the cell. Obviously, the inhibitory postsynaptic potential cannot be due to the same ionic changes, because during inhibition the potential becomes more negative. One must assume either movements of positive ions out of the cell or negative ions into the cell. This could be outward movement of a cation such as potassium or the inward movement of anion such as chloride, or the combined movements of ions in their respective direction. All these ionic changes increase the small internal excess of anions giving rise to hyperpolarization.

Since the resting cell membrane is reasonably permeable to potassium and chloride although sparingly to sodium, there is a potential difference across the membrane

of such nature that the axoplasm is negative with respect to the extracellular fluid. The redistribution in order to equalize is opposed by the influence by the negative charge within the fiber. There are several theories how the concentration gradient originatesand how the resting potential is produced. The recent one is the "sodium pump", which transports sodium out and potassium in (160). Unquestionably, metabolic processes such as enzymatic reactions are essential to membrane function. Eccles considered it necessary to postulate that specific transmitter substances like adrenaline, noradrenaline, mescaline and LSD cause the inhibitory synaptic responses by changing the ionic permeability of the sub-synaptic membrance. He could show that membranes become highly permeable to chloride and potassium while retaining a high degree of impermeability to sodium ions. He also demonstrated that there is an interplay between excitatory and inhibitory effects (161).

Hoffmann et al. studied (162) the rate and rhythm of contraction on the papillary muscle. They concluded that progressive decrease in tension ensues with increasing rate. Potassium depletion, calculated in terms of loss or gain in fiber potassium, cause weak contractions. Sodium depletion enhances the contractibility at normal rates. Adrenaline affects the force of contractions at intermediate rates. They concluded that neither endogenous nor circulating catecholamines are responsible for the basic force-frequency

curve.

Ellis demonstrated (163) that prolonged treatment with maleonate (enzyme inhibitor) depressed the heart and prevented adrenaline stimulation. This effect could be counteracted by succinate. The author speculated that the inotropic effect of adrenaline is acting on an energy supply chain rather than on a receptor. Dresel tested (164) the ino and chronotropic action of adrenaline, noradrenaline and isoproterenol. The latter thought to be naturally occurring in organs by Lockett (165) but this finding has been disproved lately by Muscholl and herself (166). Dresel found a ratio of relative potency for these catecholamines, 1-0.8-100. However, Ellis (167), Sutherland and Cori (168) indicate that the potency ratio for glucose production is 1-0.2(0.1)-0.2.

The heart, as other visceral organs, is innervated through both the sympathetic and parasympathetic nervous systems, which are synergistic in function. Consequently, any disturbance of the functional balance must result in visceral dysfunction of some degree. Early in 1892, Van Noorden recognized this (169) and called attention to various clinical conditions associated with increased vagus irritability, distinguishing the vagotonia and sympathicotonia.

Babkin in 1932 advanced certain data (170) in support of the assumption that the complex balance of excitation and inhibition exhibited by autonomic nerves in mammals represents a recent evolutionary development. In lower species, according

to Östlund and himself (171) the autonomic nervous system subserves only excitatory functions.

g) Effect of Hormones

1. Thyroid Hormones

It has been long known that some kind of interrelation exists between the catecholamines and the thyroid hormones. More direct indications of such a relationship have been obtained for the toxicity of adrenaline, which increased after feeding the test animals with thyroid (172, 173). The hyperglycemic action of catecholamines was increased during the first 3 days by feeding the animals with thyroid, but after this period the sensitization subsided so that after 14 days the effect was less than before thyroid feeding.

2. Adrenal Cortical Hormones

An influence of the adrenal cortical hormones on the activity of catecholamines has also been demonstrated. Injections of noradrenaline in the adrenalectomized dog give progressively smaller pressor responses as the blood pressure falls to shock levels. A single injection of adrenocortical extracts then potentiates the blood pressure response to previously ineffective concentrations of noradrenaline in adrenalectomized dogs but not in normal controls. DCA was ineffective in this respect (174). The blood pressure response to noradrenaline is less altered than to adrenaline in adrenalectomized dogs maintained on DCA without an accompanying high salt diet.

Raab, Humphreys and Lepeschkin (175) found a significant rise in the average pressor effect of noradrenaline after DCA in man. This led them to postulate that such an effect might be a pathogenic factor in essential hypertension.

Fritz and Levine (176) used the rat mesoappendix preparation to observe blood flow under various experimental conditions and found that the blood vessels of the adrenalectomized rat became refractory to repeated topical applications of noradrenaline. By local application of an aqueous adrenocortical extract, the responsiveness was restored. West (177), experimenting with adrenalectomized animals, found that glucocorticoids exert a control over the levels of histamine and 5HT in animal tissues.

De Meio (178) reported differential increases of serotonin concentration in different parts of the rat brain 24 hours after adrenalectomy or hypophysectomy. Paasonen and Vogt (179), repeating his experiment under better controlled experimental conditions, failed to show that acute bilateral adrenalectomy influenced brain serotonin levels in the rat.

Cleghorn and Hall (180) first called attention to the myocardial and coronary artery damage in dogs dying of adrenal insufficiency. Cleghorn (181) concludes that the loss of adrenal medulla cannot be held responsible for the impaired vascular response in adrenal insufficiency and it is suggested that an important factor contributing to the terminal low blood pressure in adrenal insufficiency may be exhaustion of

the chemical mediator of impulses of cardio-accelerator and vasoconstrictor nerves. Cats kept in good condition by cortical extract treatment after adrenalectomy showed responses to brief stimuli and to drugs which, though exhibiting certain qualitative differences, compare favorably with results obtained on acutely adrenalectomized controls.

Cleghorn et al. (182) also reported that adrenalectomized dogs long maintained by cortical hormone treatment and not exhibiting clinical or biochemical signs of adrenal insufficiency showed an impaired cardiovascular response to adrenaline, nicotine, pitressin and barium chloride. This may have been due in part to abnormal responsiveness of the heart or vascular smooth muscle. In cats dying of adrenal insufficiency, splanchnic nerve stimulation or the intravenous injection of pitressin or barium chloride constrict blood vessels in the splanchnic region as in healthy adrenalectomized controls. The pressor response to these procedures in adrenal insufficiency was poor while that elicited by adrenaline was practically unimpaired. It was concluded that the retention of the pressor effect by adrenaline is attributable to its cardiac stimulting action. Cleghorn and Fowler (183) reported that the adrenalectomized-sympathectomized dogs did not live as long after withdrawal of cortical hormones as did others subject only to adrenalectomy. The blood chemical changes were quite marked in these animals but since death occasionally occurred with normal electrolyte levels in adrenalectomized

animals, the more or less severe changes in these constituents were hardly decisive in determining the early deaths. They concluded also that reduction in blood flow due to vaso-constriction is not a significant factor in the production of congestive changes since these were found in the sympathectomized animals dying of adrenal insufficiency as well as in the non-sympathectomized.

Insulin

The effect of hypoglycemia on the adrenal medullary secretion has been studied on several occasions. In the papers of Cannon (184) and Houssay et al. (185), it was shown that insulin hypoglycemia caused an increased secretion of adrenaline in the cat, dog and rabbit. Cannon also observed that during the hypoglycemic phase an acceleration of the denervated heart of the cat which was abolished by denervation or extirpation of adrenal gland or by glucose injection. A direct analysis of the suprarenal venous blood during insulin-induced hypoglycemia in cat was made by Duner (186) who found that the increased release was almost entirely confined to adrenaline while the increase in noradrenaline was very moderate. He also obtained evidence that a reflexogenic zone was situated in the head, probably in the hypothalamic region.

From these experiments, it is clear that the glycemic level in some way regulates the medullary secretion, a possible "chemoreflex" leads to a largely selective secretion of adrenaline while the noradrenaline secretion is comparatively unaltered. The importance of this homeostatic reflex is also

illustrated by the finding of Schlossberg et al. (187) that the spontaneous recovery after a dose of insulin was slower or absent in sympathectomized animals, whereas normal animals showed a spontaneous recovery after the blood sugar had fallen to the level of hypoglycemic symptoms.

A depletion of the adrenal gland after insulin hypoglycemia has been noticed by several authors. In an intensive study on rats, Hökfelt (188) was able to show that depletion after large doses of insulin became almost complete for adrenaline but considerably less for noradrenaline.

3. Effect of Removal of Endocrine Organs

Hokfelt has studied extensively (188) the effect of the removal of endocrine organs and the administration of hormones on the level of catecholamines in different organs.

h) Reserpine

Despite ancient belief in the curative powers of snakeroot in India, nearly 2,500 years passed before the Western world heard of it. In the 16th century, a German physician named Leonhard Rauwolf traveled through many lands gathering native medical plants. His journey did not carry him through India and it is a question whether he himself actually saw this plant which "cured madness". When the snakeroot plant reached Europe some years later, the French botanist Plumier named it in his honor, Rauwolfia, because of his bravery in reaching out for new knowledge through

perils and dangers.

Not until the 20th century, after hundreds of thousands of Indian patients had been treated with rauwolfia root for numerous ailments, did a few careful Indian botanists and clinicians conduct tests on the plant and its powers. Soon after, scientists in Switzerland and in the United States undertook a thorough investigation, extracted and purified the valuable drug, which has exerted a remarkable influence upon the modern clinical research, practice, and basic understanding. It was noticed that when reserpine is given, the final breakdown product of serotonin, 5-hydroxyindolacetic acid appears in increased concentration in the urine. When animals received a further injection of reserpine, the urinary excretion of 5-hydroxyindolacetic acid remains normal. Carlsson et al. (189) proposed that the central actions of reserpine are actually mediated through serotonin "antagonism". After intravenous injection of reserpine to rabbits, the drug rapidly enters the brain, achieving its maximal concentration in about 10 minutes. The brain level then declines rapidly and in about 2 - 4 hours, reserpine cannot be detected. While the brain levels are declining, the pharmacological effect progressively increases, becoming maximal in 2 - 4 hours. This occurs at a time when serotonin levels are particularly low. The effect persists for 2 days, then the level of serotonin increases and the pharmacological effect diminishes. Thus certain actions of reserpine are related in time to the

change in concentration of brain serotonin and not that of reserpine.

In addition to serotonin, reserpine depletes the tissues of catecholamines, such as adrenaline, noradrenaline. Shore et al. (190) proposed that reserpine released catecholamines from their binding and the unstable form metabolized. Catecholamines continue to be synthetized in the body and the low level persists till the binding sites regain their binding capacity or new sites are formed. It represents a balance - counterbalance between rate of synthesis and rate of metabolic transformation. It has been observed by Vogt (191) that after reserpine administration, the sympathetic cardiac nerves failed to respond to stimulation. Administration of the drug acts like a chemical denervation. It has been shown by Bertler, Carlsson and Rosengren (192) that reserpine administered intravenously was found to cause almost complete disappearance of catecholamines from the heart of the rabbit. Paasonen (193) and several others repeatedly demonstrated that administration of reserpine led to depletion of heart muscle of adrenaline and noradrenaline in all mammals studied. It has been demonstrated by Krayer (194) that the drug when given in a single dose to the heart - lung preparation, causes a definite increase in the heart rate of the isolated organ. This effect is similar to a continuous infusion of adrenaline or noradrenaline. In principle, the effect of reserpine on the catecholamine content of the heart is very

similar to its action on the brain serotonin content, inasmuch as the action of reserpine outlasts the detectable presence of reserpine and the pharmacological action of reserpine is due to the release of amine.

Waud (195) found that reserpine in doses ranging from 0.03-3.0 mg/l in single dose will deplete the heart so as to make a challenging dose of 3.0 mg/l reserpine ineffective in a heart - lung preparation 24 hours later. The depletion reaches its maximum in 24 hours, remains there for 3 - 6 days. Repletion requires 10 - 14 days. Accordingly, with repeated doses, much lower quantities are required. By calculation, 10 daily doses of 0.21 mg reserpine in a 70 kg. man will deplete the heart. Muscholl (196) observed that reserpine caused a similar loss of catecholamines from all parts of the heart. The concentration of noradrenaline was reduced by 93-97%, the adrenaline 59-79% with a single injection of 1.8 mg/kg reserpine i.p. at 18 hours. These observations suggest that the adrenaline of the heart behaves differently from that of the sympathetic ganglia or post-ganglionic nerve fibers, in which the adrenaline concentration is not significantly affected by reserpine. Ashwin (197) administered reserpine to adrenalectomized rats maintained on 1% sodium chloride and regular diet. He noticed that death of the animals occurred within 6 hours. Adrenalectomized rats receiving 2.5 mg/kg of DCA did not show these features after reserpine.

Burn, Macmillan and Rand (198) advanced a theory recently that the increased sensitivity to adrenaline and noradrenaline seen in:

1. sympathectomized structures,
2. tissues treated with reserpine,
3. animals after intravenous administration of cocaine,

is due to the fact that under these three conditions, amines which are not derivatives of catechols, such as tyramine and phenylethylamine, lose their action on the heart and other organs. Following denervation of reserpine, the noradrenaline content of these tissues is depleted. These observations suggest that substances like tyramine normally act by liberating noradrenaline. In the case of reserpine, there are no catecholamines to liberate; in the case of cocaine, it prevents the release of catecholamines from the nerve endings. Thus the store of noradrenaline in sympathetically innervated tissues appear to play a part in normal physiology.

III. METHODS AND MATERIALS

A. Animals

Adult male albino rats, Sprague-Dawley strain, weighing between 80-100 gm., obtained from a local dealer were used for adrenalectomy and employed as the source of tissue for other experiments.

B. Treatment of Animals

The rats were divided by random procedure according to Fisher (199) into experimental groups.

Adrenalectomy was carried out under ether anesthesia, via lumbar route in one stage bilateral operation.

Sham operations on control animals were carried out but instead of adrenalectomy, a small piece of periadrenal fat was removed and the incision closed.

The animals were weighed post-operatively and at the end of the experiments.

a) Diets

The usual diet was Purina Fox Chow ad libitum and tap or glass distilled water as indicated. Sodium deficiency was achieved by maintaining the rats on "NBC sodium deficient diet", devised according to the modification of Itter et al. (200).

Composition:

Sucrose	72%
"Vitamin free" casein	18%
Butter fat (salt free)	5%
Sodium free salt mixture	5%

Composition of sodium free salt mixture:

Calcium carbonate	37.5%
Pot. Phosphate monobasic	28.4%
Potassium chloride	18.9%
Ferric citrate	9.5%
Magnesium oxide	3.8%
Copper sulphate	1.9%

The animals received diet ad libitum and glass distilled water for drinking. Control animals received 1%

sodium chloride mixed in the diet, deficient animals 1% corn starch in place of the salt.

b) Drugs

Ether U.S.P.X. Merck (Ethyloxide U.S.P.)

Reserpine, Lot No. 8183 - 8469

SERPASIL, donated by the "CIBA" Co. Ltd. in vials of 2 ml. containing 5 mg. of the drug. Control animals were injected with "Placebo" (Serpasil Placebo - CIBA) Lot No. 5792 in the same manner and similar amounts as to the drug.

SERPASIL was diluted with "Placebo" prior to injecting it, and dilution was freshly prepared before each experiment.

IPRONIAZID was donated by the Hoffmann - La Roche Ltd. Lot No. R595 and the powder form was dissolved in 0.2 M. phosphate buffer pH 7.4. The control animals were injected with saline.

Desoxycorticosteron Trimethyl-acetate was donated by "CIBA" Co. in ampules containing microcrystals in suspension Lot No. 9236491018. Hydrocortison was given by courtesy of Merck Laboratories in alcohol-free, injectable suspension.

In some experiments DCA powder was used, donated by Dr. Birmingham, dissolved for injection in propylenglycol.

c) Chemicals

Most of the chemicals used were purchased from the Fisher Scientific Company but adrenaline, noradrenaline and dopamine were obtained from Winthrop-Stearns, Incorporated.

C. Experimental Procedure with Adrenalectomized Animals

The adrenalectomized animals and sham operated controls were subjected post-operatively to various diets and drug treatments for the periods of time as indicated in the Tables showing the results of the individual experiments.

Tissues for extraction were prepared as follows: The animals were killed at the times indicated on the experimental schedules (see Tables) by cervical fracture or by decapitation and bleeding from the neck vessels. Post-mortem, the completeness of adrenalectomy was checked by examining the adrenal sites. Then, organs were quickly removed, rinsed free of clotted blood and blotted on filter paper. In all cases, the heart was removed, several cuts were made into the muscle and rinsed to remove any clotted blood that had been trapped inside the heart. The organs were then weighed, minced with scissors in 10% trichloracetic acid and the catecholamines extracted within hours, or the tissues wrapped in paraffin films and placed in the deep freeze until the extractions were to be done. The homogenization of organs was done by the VIR-Tis "45" homogenizer in 10% trichloracetic acid (TCA), using high speed in accordance with the consistency

of the organ. For most of the organs, 10 ml. of TCA was used, for the adrenals, 2 ml. of TCA was added and the extract made up to 50 ml. with 10% TCA and 5 ml. aliquots used. The homogenates then were centrifuged, the homogenates removed and the purification of catecholamines was carried out using neutral alumina. 0.5 M acetic acid eluates of 5 ml. were prepared for fluorometry.

The collection of urine was carried out using plastic metabolic-cages, where the animals were allowed to drink but not to eat. Urine was collected in 2 ml. conc. HCl and filtered, if not clear, and made up to 50 ml. with glass distilled water. After each 24 hour collection, the animals were placed back in their cages for 12 hours, where they had free access to food and fluid.

The purification of catecholamines was the same for the urine aliquots and for tissue homogenates as follows: Into the test tube, first 5 ml. of 10% Versene and one drop phenolphtalein, then 2 gr. of neutral (non-alkaline aluminium oxide Woelm, activity grade 1) alumina was added. The alumina was necessarily of chromatographic grade. The solution was neutralized by addition of 20% sodium hydroxyde with constant swirling of the suspension. The pink indicator color became stable only gradually since acid is slowly released from the alumina. The test tubes were shaken for 5 minutes vigorously (mechanical agitator), then stopped and more alkali added as necessary to restore the indicator color and the 5 minute shaking repeated. The pink color was usually stable by this time.

The alumina was then allowed to settle down and the supernatant removed by suction (water pipe) applied through a fine-bore pipette. Five ml. of glass distilled water was added to the alumina and the procedure of shaking (manually) and removal of the supernatant repeated. This procedure had to be carried out twice. For the elution, 10 ml. of 0.5 M acetic acid was shaken with the alumina for 15 minutes, at the end of which time, the supernatant was decanted into a test tube, centrifuged and preserved for estimation. The procedure could be interrupted at this stage and the eluates held in the refrigerator until required.

a) Fluorometry

In our laboratory, fluorescence of the solution is measured with the Aminco-Bowman Spectrophotofluorometer, containing the 1P21 phototube. Estimating adrenaline and noradrenaline, a Corning 3385 (yellow) filter was inserted in the instrument, selecting the proper wavelength for these components. The activation and fluorescence maxima of the catecholamines as found in our laboratory is shown on Table I.

b) Procedure for Estimating Adrenaline

Two aliquots, 0.5 and 1 ml. respectively, of each eluate were used. The first was made up to 1.0 ml. with 0.5 M acetic acid, then 2 ml. of buffer Glycine pH 3 was pipetted in. For the oxidation, 0.5 ml. of iodine solution, was added. The iodine solution, 0.01 N, was diluted daily from 1.0 N with glass distilled water. At the end of exactly 3 minutes, 0.5 ml.

of thiosulphate solution was added. The tubes were then shaken vigorously to dispel excess iodine. At the end of another 3 minutes, 1 ml. of sodium hydroxide-ascorbic acid was added in order to isomerize the obtained chromes to the lutins and to insure stability. After a waiting period of 15 minutes, the solution was read on the spectrophotofluorometer at the appropriate wavelength. A fresh sodium hydroxide-ascorbic acid solution was always prepared before each reading by dissolving 2 mg. of ascorbic acid for each 1 ml. of 5 N NaOH.

c) Procedure for Estimating Noradrenaline

The same principles and procedures as described above for adrenaline were utilized but differential oxidation was carried out using IM Sodium acetate buffer pH 6. After the addition of the sodium hydroxide-ascorbic acid solution, the waiting period was 45 minutes, then solutions were read at the appropriate wavelength.

d) Procedure for Estimating Dopamine

This was done according to the technique of Sourkes and Murphy (65) to the 1 ml. aliquot of alumina eluate from each sample, 2 ml. of 1 M, pH 6.0 acetate buffer was added and oxidation carried out by the addition of iodine as above. At the end of 3 minutes, 0.5 ml. of 4.5 N NaOH containing 12.6 mg. anhydrous sodium sulphate was added. In a further 3 minutes, 1 ml. of 5 N hydrochloric acid containing 2 mg. ascorbic acid was added and after a 45 minute waiting period, the solutions were read on the spectrophotofluorometer.

e) Recovery of the Catecholamines

Since recovery of added catecholamines is not complete, suitable corrections must be made in evaluating the figures obtained. To 3 samples of aliquot to be determined in the run, or to the same amount of 10% trichloracetic acid used for extraction, known amounts of catecholamines, 1, 2 and 3 µg respectively were added. These samples were treated exactly as the others and the amount of catecholamine present determined. The corrected galvanometer readings by calculating the "µg of catecholamine added" vs. "µg of catecholamine recovered" were used to convert the readings for the unknowns directly to "estimated catecholamine".

f) Blanks

At the same time as the eluates were run, a blank was prepared. By estimating tissue extracts it contained the same amount of 10% TCA as used for extraction; in the case of urine, it consisted of "pooled urine". In estimating adrenaline and noradrenaline, the tubes were treated in the same fashion as the others. In estimating dopamine, the oxidation step was omitted.

g) Standard Curve

For each estimation, a standard curve was prepared using 0.1-0.2-0.3 µg catecholamine respectively. In estimating noradrenaline, a recovery and a standard curve was prepared for adrenaline as well. Adrenaline oxidized at pH 6 was used as a correction factor in estimating noradrenaline.

h) Calculations

All galvanometer readings were corrected for the deflection observed with the reagent blank. From the standard curve, a factor, F, is calculated as F mm. scale length deflection per μg of catecholamine. The average catecholamine content estimated using the two aliquots was taken as the result.

D. Statistical Analysis

Symbols used were:

X = observed value of a variety which is measured

n = number of item measured

$\bar{x}$ = means = $\frac{SX}{n}$

V = Variance = (Standard Deviation)^2

S.E. = Standard Error = Standard Deviation of the Mean

D.F. = Degree of Freedom = number of Degrees of Freedom = n-1

($1^{o}F$ lost because of the mean)

Calculation to get S.E.

I. Sum of Squares of Deviations from the Mean =

= $S(x_2) - \overline{x}Sx = Sx^2$

II. V = Variance = $\frac{Sx_2}{n-1}$

III. Standard Deviation (of a sample) = $\sqrt{V}$

IV. Variance of the Mean = $\frac{Sx^2}{(n-1)n} = Vx^2$

V. Standard Error (or Standard Deviation of the Mean) = $\sqrt{V\bar{x}}$

Calculation to get S.E. of Difference:

I. Mean ± S.E.

		D.F.
$\bar{x}_1 \pm S_1$	n_1	n_1-1
$\bar{x}_2 \pm S_2$	n_2	n_2-1

Difference = $\bar{x}_2 - \bar{x}_1$

II. S.E. of the Difference = $\sqrt{S_1^2 - S_2^2}$

III. Degree of Freedom = $n_1 - n_2 - 2$

Calculation of V of the population. (Analysis of Variance)

I. $S(x^2)$

II. $\frac{(Sx)^2}{n}$

III. $S(x^2) - \frac{(Sx)^2}{n}$ - Sum of Squares (of the deviations from the mean) = S of S (for the total) = $S(x_2)$ = xSX - Correction Factor

IV. $V = \frac{\text{S of S}}{n-1}$

V. Same calculation as above for the sub-groups, using their sums.

= S of S (for groups)

VI. Variance of the mean difference - $\frac{V_1}{n_1} - \frac{V_2}{n_2}$

VII. S.E. = $\sqrt{\frac{\text{S of S}}{n(n-1)}}$

VIII. $t = \frac{\bar{x}}{\text{S.E.}}$ - necessary t 5% for significance

IX. Set up a table:

S of V	D of F	Cor.S of S	Mean Squares-V-$(SD)^2$	F ratio
All	n-1	0000000	-	-
Between groups	1	0000000	00000000	>1.0
Remainder	n-2	0000000	00000000	< 1.0

Calculations to get P for the Means:

I. Difference between Means

II. S.E. of the Difference - $\sqrt{\bar{V}x_1 - \bar{V}x_2}$

III. $t = \dfrac{\bar{x}_1 - \bar{x}_2}{\text{S.E. of diff.}}$

IV. P = significant if >0.05 (201)

IV. EXPERIMENTAL RESULTS

A. Effect of Adrenalectomy, Dietary Sodium, with or without Administered Cortical Hormones on the Catecholamine Content of the Rat Heart and Brain

Normal values for catecholamines in the organs of a 100 gr. rat were found as follows:

values are Mean' ± S.E./total organ

	Adrenaline(A)	Noradrenaline(N)	Dopamine(D)
Heart	0.020	0.372±0.014	-
Brain	-	0.403±0.013	0.512±0.012
Spleen	-	0.242±0.009	-
Kidneys	0.002	0.102±0.012	-
Liver	0.014	0.130±0.016	-
Adrenals	18.8±2.4	3.41±0.49	(A - N ratio 21%)

These values agree well with other authors' findings (142, 188), however, the values for brain noradrenaline was found to be somewhat lower than reported by others. It is a general opinion that the values for adrenaline are questionable due to the uncertainty of the method for adrenaline because it is believed that the fluorescence emitted by a solution to be estimated for adrenaline is composed of scattered light and that there is also interference with dopamine fluorescence.

Five experiments were set up to study the effect of adrenalectomy combined with sodium deficiency on the catecholamines of the heart. Rats ranging between 80-100 gr. were

used. The animals were maintained pre-operatively on Purina Fox Chow and tap-water ad libitum. Adrenalectomy and sham-operations were carried out as described previously. Animals were then allowed to recover and put in cages and placed immediately on diets. One group of rats was maintained on Purina Fox Chow and tap-water ad libitum. Sodium deficiency was achieved by maintaining another group on NBC "sodium deficient test diet" and glass distilled water ad libitum. One of the most striking features of these adrenalectomized animals, receiving no replacement therapy with cortical hormones, was the sudden death which occurred from the immediate post-operative period up till several days afterward. We encountered considerable difficulty experimenting with such treated animals and lost 58% of our adrenalectomized animals as death usually occurred at night. However, we had the opportunity to observe closely the death of six rats.

Some hours before death occurred, the animals exhibited a lassitude and became "ill looking". The fur became dull and the animals had diarrhoea. Rapid breathing was very prominent. As the time advanced, the respiration slowed down and became stridulous, bradycardia ensued, salivation at the angles of the mouth and later, very thick mucus developed, obstructing the larynx. Pre-mortem, the animals developed opisthotonus and intermittently, a flaccid paralysis of all voluntary muscles. Twitching of the whole body could be induced by the least noise. Finally, at the height of a stormy fit,

death occurred. Most deaths were encountered in the group maintained on NBC "sodium deficient diet" and glass distilled water (98%). This fact forced us to change the experimental design so as to maintain this group on the deficient diet with 0.9% saline as drinking water ad libitum. The surviving animals were sacrificed at the times indicated on the experimental schedule and organs were estimated for catecholamines. Values of catecholamines obtained from similar experiments were pooled. The mean values are shown on the following Table IX.

TABLE IX

Adrenaline in heart

Days after adrenalectomy	1	3	6	9	
Ax	(15) 0.042	(15) 0.045	(16) 0.045	(14) 0.049	Purina Fox diet
	(16) 0.04	(15) 0.05	(15) 0.04	(15) 0.05	Sod. def. diet
Sham	(16) 0.051	(15) 0.054	(15) 0.047	(17) 0.04	Purina Fox diet
	(16) 0.04	(15) 0.05	(15) 0.04	(15) 0.04	Sod. def. diet

Values are mean values expressed in microgram/total organ and summarize 3 similar experiments.

Numbers in brackets show the number of animals used for the experiment.

Noradrenaline in heart

Days after adrenalectomy	1	3	6	9	
Ax	(15) 0.432 ± 0.019	(15) 0.397 ± 0.021	(16) 0.152 ± 0.018	(14) 0.167 ± 0.009	Purina Fox diet
	(16) 0.137 ± 0.012	(15) 0.171 ± 0.011	(15) 0.172 ± 0.024	(15) 0.137 ± 0.008	Sod. def. diet

Days after adrenalectomy	1	3	6	9	
Sham	(16) 0.492 ± 0.022	(15) 0.358 ± 0.017	(15) 0.362 ± 0.018	(17) 0.511 ± 0.028	Purina Fox diet
	(16) 0.291 ± 0.028	(15) 0.323 ± 0.024	(15) 0.288 ± 0.026	(15) 0.285 ± 0.006	Sod. def. diet

Values are mean values, expressed in microgram/total organ.

Numbers in brackets show the animals used for the experiment.

From these results, it appears that adrenalectomy leads to a decrease in the cardiac catecholamine content. The decrease at 7 - 9 days following the operation proved to be statistically significant:

Sources of variation	D of F	Corrected S of S	Variance $(SD)^2$ mean square	F ratio
All	11	0.371789	----	
Between group	1	0.356385	0.356385	231.42
Remainder	10	0.015404	0.001540	1.0

From the following table, it appears that the sodium deficiency as such, with or without adrenalectomy contributes to a drop in the catecholamine level. The values for adrenalectomized animals declined with time if on Purina Fox Chow diet, but the values were low from the first day if the animals were placed on the deficient diet.

Noradrenaline in heart

(Values are not true values, but are summarized from the previous table)

Days	1	3	6	9
Ax	0.28	0.28	0.16	0.15
Sham	0.39	0.34	0.32	0.40
Purina Fox	0.46	0.38	0.26	0.34
Sod. def.	0.21	0.25	0.28	0.21

On account of these findings, we attempted to clarify

the effect of dietary sodium deficiency combined with adrenalectomy upon the catecholamine content of the heart and other tissues, particularly, the brain. Our specific aim was to elucidate whether or not the sodium withdrawal could influence the level of catecholamines in the tissues if the animals were pre-treated with cortical hormone, namely, desoxycorticosterone, post-operatively.

Rats, weighing 80-100 gr., were adrenalectomized and sham operated respectively. The animals were assigned to diets as shown and given distilled water ad libitum.

	Adrenalectomized		Sham operated	
	Set I	Set II	Set I	Set II
NBC "sodium def. diet" + 1% corn starch	25%	25%	25%	25%
NBC "sodium def. diet" + 1% NaCl	25%	25%	25%	25% (of treatment group)

Post-operatively and thereafter, the animals were injected daily with 0.5 mg/100 gr. body-weight DCA subcutaneously. Set I was sacrificed at 2 days post-operatively by decapitation, Set II on the 6th post-operative day.

Table X shows the values of catecholamines found in the heart, brain, spleen, kidneys and Table XI contains the mean values of these. Examination of the values obtained for the heart and brain shows that adrenalectomy induced a drop in the level of noradrenaline in the heart at 2 days post-operatively, but there was no further drop with time. Practically the same low level could be detected on the 6th post-operative day. In

Table X

Results

Set. 1

Diet	Adrenalectomized heart A	heart AN	brain N	brain D	spleen N	kidney A	kidney N	Sham operated heart A	heart N	brain N	brain D	spleen N	kidney A	kidney N	adrenals A	adrenals N	mg/gr fresh tissue
	0.01	0.227	0.384	0.454	0.184	0.001	0.009	0.02	0.327	0.358	0.403	0.215	0.001	0.09	0.61	0.11	
-Na	0.01	0.184	0.402	0.508	0.272	0.009	0.11	0.01	0.284	0.272	0.384	0.233	0.001	0.12	0.58	0.08	
	0.02	0.203	0.307	0.472	0.193	0.007	0.11	0.02	0.292	0.191	0.275	0.252	0.005	0.10	0.77	0.14	
	0.01	0.231	0.508	0.518	0.218	0.004	0.14	0.02	0.303	0.404	0.508	0.241	0.004	0.14	0.65	0.11	
Na	0.02	0.212	0.442	0.572	0.815	0.002	0.08	0.02	0.278	0.285	0.362	0.273	0.003	0.11	0.91	0.18	
	0.02	0.228	0.333	0.501	0.227	0.006	0.09	0.02	0.365	0.372	0.417	0.201	0.003	0.12	0.73	0.16	

Set 2

	0.01	0.184	0.307	0.333	0.207	0.001	0.08	0.02	0.354	0.335	0.411	0.184	0.003	0.10	0.68	0.12	
-Na	0.01	0.171	0.408	0.392	0.188	0.002	0.07	0.01	0.336	0.382	0.432	0.302	0.001	0.10	0.82	0.14	
	0.01	0.292	0.309	0.401	0.292	0.002	0.12	0.03	0.372	0.323	0.383	0.237	0.002	0.08	0.55	0.07	
	0.01	0.204	0.353	0.372	0.267	0.001	0.16	0.03	0.284	0.401	0.455	0.250	0.002	0.07	0.84	0.18	
Na	0.03	0.188	0.404	0.484	0.245	0.000	0.08	0.02	0.377	0.342	0.392	0.244	0.002	0.08	0.92	0.15	
	0.01	0.235	0.485	0.508	0.283	0.001	0.10	0.02	0.341	0.315	0.320	0.232	0.003	0.12	0.88	0.15	

Values are given in g./total organs (pair of kidney's and adrenals).

TABLE XI

Set I

	Adrenalectomized							Sham Operated								
	heart		brain		spleen	kidney		heart		brain		spleen	kidney		adrenals	
	A	N	N	D	N	A	N	A	N	N	D	N	A	N	A	N
-Na	0.013	0.204	0.364	0.478	0.218	0.056	0.10	0.016	0.301	0.273	0.354	0.266	0.002	0.10	0.65	0.11
+Na	0.016	0.223	0.427	0.530	0.220	0.040	0.07	0.020	0.315	0.353	0.428	0.238	0.003	0.12	0.76	0.15

Set II

-Na	0.010	0.215	0.341	0.341	0.226	0.016	0.08	0.020	0.354	0.346	0.408	0.241	0.002	0.09	0.68	0.11
+Na	0.016	0.209	0.414	0.414	0.265	0.006	0.11	0.023	0.334	0.352	0.389	0.242	0.002	0.09	0.88	0.12

Values are given in microgram/total organ

TABLE XII

	HEART		BRAIN					
Diet	A		N		N		D	
	AX	SHAM	AX	SHAM	AX	SHAM	AX	SHAM
means ± SE	0.011	0.018	0.210±0.018	0.327±0.014	0.353±0.024	0.310±0.028	0.427±0.027	0.381±0.023
-Na range	0.01-0.02	0.01-0.03	0.171-0.292	0.284-0.372	0.307-0.408	0.191-0.382	0.333-0.508	0.275-0.432
means	0.016	0.022	0.216±0.024	0.325±0.017	0.421±0.029	0.353±0.020	0.492±0.024	0.409±0.028
+Na range	0.01-0.03	0.02-0.03	0.188-0.235	0.278-0.377	0.333-0.508	0.285-0.404	0.372-0.572	0.320-0.508

DOC — 0.5 mg. daily

values are means, µg./total organ.

TABLE XIII

Effect of adrenalectomy and dietary sodium on heart noradrenaline.

Table of P values.

Ax. : sham

	S.E. of difference	D of F	t	P
-Na	± 0.023	10	5.09	>0.01
+Na	± 0.029	10	3.76	>0.01

sodium

Ax	± 0.030	10	0.20	< 0.05
sham	± 0.022	10	0.12	< 0.05

Effect of adrenalectomy and dietary sodium on brain noradrenaline.

Table of P. values

Ax. : sham

	S.E. of difference	D of F	t	P
-Na	± 0.034	10	1.26	<0.05
+Na	± 0.035	10	1.94	<0.05

sodium

Ax.	± 0.035	10	1.94	<0.05
sham	± 0.034	10	1.26	<0.05

Effect of adrenalectomy and dietary sodium on brain dopamine.

Table of P values

Ax. : sham

	S.E. of difference	D of F	t.	P
-Na	± 0.035	10	1.31	<0.05
+Na	± 0.037	10	2.24	<0.05

Sodium

Ax.	± 0.036	10	1.80	<0.05
sham	± 0.036	10	0.77	<0.05

consideration of this, we prepared Table XII which shows the mean values for the two experiments, not respecting the time after the operation. This table was submitted to statistical analysis to be found in Table XIII.

This analysis proved that there exists a significant decrease in the catecholamine (noradrenaline) content of the heart of the adrenalectomized animals as compared with sham operated controls. It is also shown that the dietary sodium withdrawal did not have a significant influence upon this catecholamine level either in adrenalectomized or in sham operated animals. The values for the sodium deficient animals tended to be somewhat lower but not to a statistically significant extent.

No change was found in brain noradrenaline after adrenalectomy, however, the brain dopamine increased somewhat in the adrenalectomized group in contrast to the sham operated animals, but it was noted only when the animals were supplemented with sodium in the diet. It proved to be statistically significant (50%) and we decided to reinvestigate it

The effect of sodium withdrawal on the catecholamine content of the adrenal gland was next studied. Using the sham operated animals, at the time of killing, we removed the adrenals and performed an estimation for catecholamines. The results are shown below:

	A	N		%N	S.E (of difference)	D.F.	T	P
-Na	14.45	2.2	2.42	0.49	16			
					adrenaline 3.26	10	1.11	<0.05
+Na	18.08	2.4	3.41	0.44	21			
					noradrenaline 0.66	10	1.50	<0.05

/pair of adrenals S.E.

From the analysis of data, it appears that the sodium withdrawal had no significant effect on the catecholamines of the adrenal gland.

By comparing the above experiments, it becomes apparent that the animals having no hormone therapy with DCA deplete their cardiac catecholamines progressively with time. If the animals were injected with DCA daily, after an initial drop, there was no further decrease with time. In order to gain further information concerning this factor, we designed an experiment giving a single injection of DCA 0.5 mg/100 gr. BW. post-operatively to adrenalectomized and sham operated controls respectively. Having established that sodium has no effect upon the ensuring catecholamine level, we maintained the animals during the whole experiment on Purina Fox Chow and tap-water ad libitum. The first group of animals was sacrificed at 24 hours after the operation, the other on the 7th day. Heart and brain were removed and the catecholamines determined in them as before. - Table XIV.

When studied statistically, it became evident that adrenalectomy caused a significant decrease in the level of noradrenaline in the heart as compared to the sham operated controls. It is also shown that there appears a further decrease in this catecholamine level on the 7th post-operative day, comparing the values of the adrenalectomized rats for the 24 hour experiment with the 7 day experiment. However, there was a significant decrease in heart noradrenaline level of sham

TABLE XIV

TABLE OF MEANS

	HEART.		BRAIN.	
	adrenaline	noradrenaline	noradrenaline	dopamine
ADRENALECTOMIZED		24 hours.		
	0.03	0.299 ± 0.022	0.403 ± 0.021	0.488 ± 0.016
	0.02	7th day. 0.213 ± 0.007	0.398 ± 0.013	0.515 ± 0.011
	HEART.		BRAIN.	
	adrenaline	noradrenaline	noradrenaline	dopamine
SHAM OPERATED		24 hours.		
	0.03	0.361 ± 0.014	0.401 ± 0.016	0.484 ± 0.015
	003	7th day. 0.230 ± 0.012	0.403 ± 0.013	0.525 ± 0.011

Mean values are in microgram/total organ ± S.E.

Statistically significant:

		S.E.±(diff.)	t	D.F.	P
I.	Noradrenaline of the heart, adrex. vs. sham - at 24 hours	0.026	2.38	17	>0.05
II.	" " " , adrex. - 24 hours vs. 7th day	0.023	3.74	14	>0.05
III.	" " " , sham - " " "	0.019	6.89	18	>0.05

operated animals also from 24 hours to the 7th day, comparing the values of the sham operated rats for the 24 hour experiment with the 7 day experiment. At this time, we could not detect any significant change in the brain catecholamines and we were unable to confirm or repeat the previously noticed increase in the brain dopamine following adrenalectomy.

Summarizing these experiments, it can be stated that adrenalectomy induced a decrease in the level of noradrenaline in the heart regardless of test diets which increased with time if no steroid therapy. The values for adrenaline in the heart were rather variable; however, no significant change was observed following these procedures. If the animals were injected with DCA daily, there was, after an initial post-operative decrease, no further decrease with time. The initial low level persisted for the entire period of the experiment. Further experiments showed that a single post-operative injection of DCA was ineffective in preserving catecholamines or noradrenaline in the heart, the initial decrease being followed by a further decrease with time. We could not obtain any convincing evidence for alteration in brain catecholamine level following adrenalectomy.

B. Reserpine Depletion of Catecholamines in Rat Tissue and the Effect of the Parallel Administration of Iproniazid

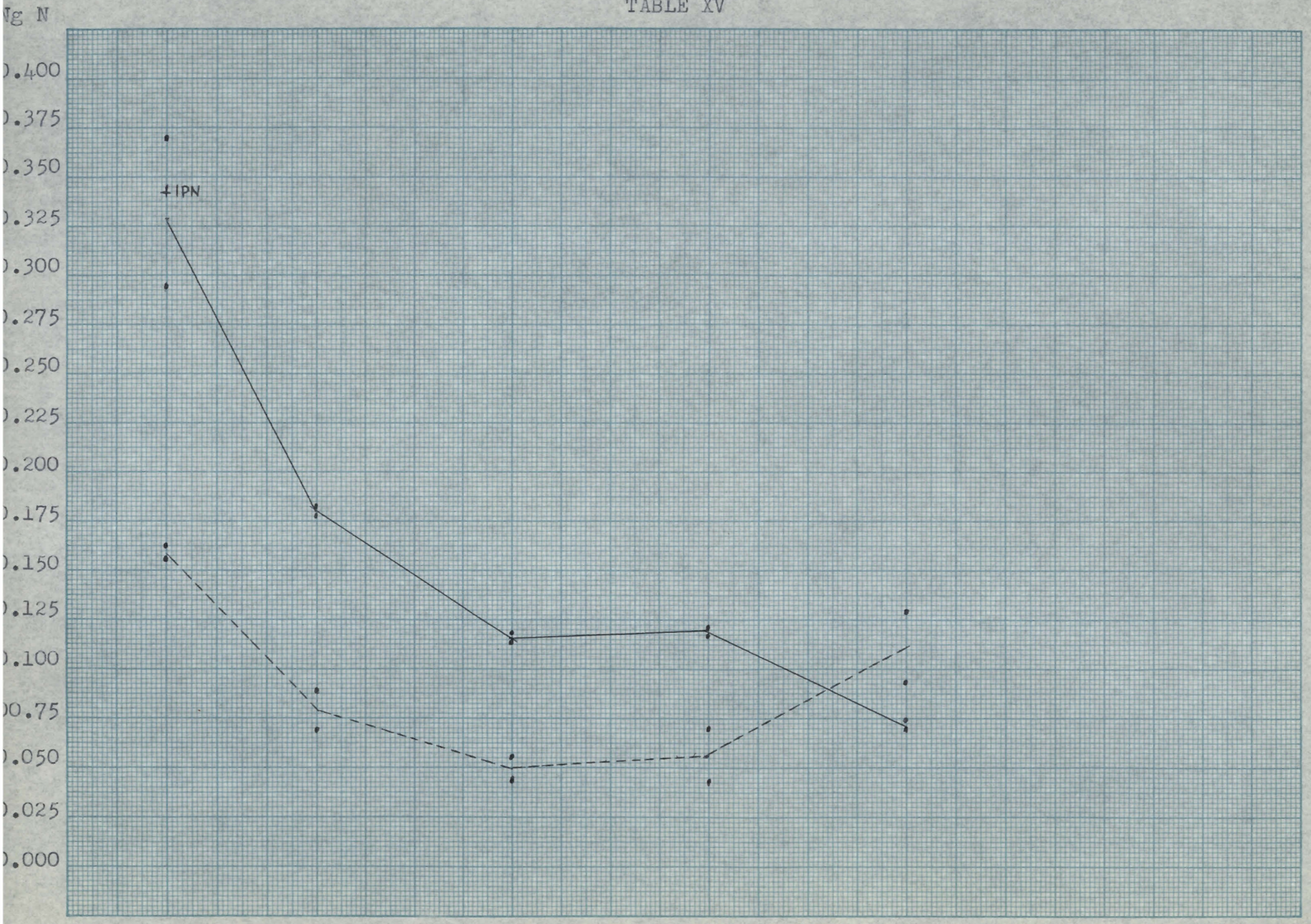
Adult male rats, maintained on Purina Fox Chow and tap-water, were injected with reserpine in the doses of 0.37 1.1-3.3-10 mg/kg. body-weight respectively. Six hours prior to

the reserpine administration, the same animals had been injected with iproniazid in a dose of 100 mg/kg. body-weight. The control group received saline in place of the iproniazid. All were sacrificed 48 hours after the reserpine administration.

Table XV shows the effect of these combined treatments on the noradrenaline of the heart. The curve is plotted as noradrenaline estimated in the heart (total organ) against dose of administered reserpine. It can be seen that reserpine induced a depletion of this catecholamine in 48 hours and that of the decrease became more prominent, though not very significantly, by increasing dose of reserpine. A single dose of 0.37 mg/kg. reserpine is effective in producing a drop in noradrenaline level of the heart, but for complete depletion, probably, doses ranging from 3.33-10 mg/kg. are required. The elevation of the curve for the 10 mg. dose is probably due to an experimental error, one of the samples estimated in this batch had an extremely high value for noradrenaline which completely altered the mean value for this group.

Iproniazid raised the values for noradrenaline in each including the placebo injected groups. However, the curve parallels with that of reserpine-saline injected group, that is, if reserpine is administered, the depletion is induced but a pre-treatment with iproniazid resulted in higher values for each treatment group, which are still lower than those of normal values. If the placebo-saline injected values were taken as normal values, it

TABLE XV



becomes evident that iproniazid raised the noradrenaline content of the hearts of these rats. By injecting 0.37 mg/kg. reserpine + iproniazid, this level drops sharply but it is still in the neighbourhood of normal levels. Increasing the dose of injected reserpine to 1.11 mg/kg., the value drops somewhat below "normal", but higher than that of reserpine-saline group values. It is apparent from this that iproniazid could combat effectively, but not completely, with the action of administered reserpine giving in increasing dose.

C. Effect of Reserpine and Dietary Sodium on the Catecholamines of the Heart and Brain

Previously, we established that adrenalectomy caused a decrease in catecholamine content of the rat heart. Our interest turned to reserpine which is known to deplete the catecholamines of the animal organs (202, 203). According to this, a pre-treatment dose of 2-10 mg/kg. will deplete the heart if given as a single injection, the depletion reaches its maximum in 24 hours and remains there from 3 to 6 days. The repletion was found to require 10 - 14 days. With repeated doses, much lower quantities are required (204). Our specific aim was to combine adrenalectomy and reserpine, both having a similar depleting action on the heart catecholamines, in order to elucidate some of the characteristics of their action and the effect played upon this by dietary sodium and steroid hormones, namely, DCA. Firstly, we designed an experiment to study the repletion of catecholamines

and the influence of dietary sodium after a single injection of reserpine. All the rats were injected with 10 mg/kg. reserpine subcutaneously. They were then divided in two groups at random and assigned to test diets. Sodium deficiency was achieved as before, by maintaining the animals on NBC "sodium deficient diet" + 1% corn starch in the food. Controls had the same diet with 1% NaCl in the food. Both groups were given glass distilled water ad libitum to drink. Half of each group was sacrificed on the 6th day after the injection, the remaining half on the 12th day.

The injected animals exhibited, in the first 4 days after the injection, diarrhoea initially, lassitude and dull fur. They became tranquilized. The tachycardia observed after the injection for 12 hours changed to bradycardia which persisted up till 7 - 9 days.

Tables XVI and XVII show the obtained values for the catecholamines of the heart and brain together with their statistical analysis. There was a significant increase in the heart adrenaline and noradrenaline between 6 and 12 days. The effect of dietary sodium withdrawal was not significant under these experimental conditions. A significant increase could be demonstrated for the brain noradrenaline and dopamine from 6 to 12 days. The effect of sodium withdrawal proved to be significant for the noradrenaline, but insignificant for the dopamine. The values for noradrenaline in the sodium deficient group were somewhat lower than those of sodium supplemented group on the 6th day,

TABLE XVI

Repletion of adrenaline and noradrenaline and influence of dietary sodium in the rat heart after a single injection of reserpine.

Table of Means.

Diet	Days after treatment. 6 (adrenaline)	12 (adrenaline)	6 (noradrenaline)	12 (noradrenaline)
N.B.C. sod. def.	0.001 S.E± n:6	0.016 S.E±0.001 n:8	0.079 S.E±0.095 n:6	0.280 S.E±0.083 n:8
N.B.C. sod. def. + Na	0.001 S.E± n:6	0.022 S.E±0.001 n:8	0.183 S.E±0.095 n:6	0.301 S.E±0.083 n:8

Values are in microgram/total organ
N.B.C. sodium deficient diet 1% NaCl, controls had 1% corn starch in the food
glass distilled water for drinking

Reserpine: 1omg/kg body-weight s.c.

Analysis of variance.
Noradrenaline.

Sources of variation	DF	S of S	MS	F
Total(28)	27	1.392356	-	-
Days	1	0.073222	0.073222	1334
sodium	1	0.000405	0.000405	1
interaction	1	0.001938	0.001938	1
Remainder	24	1.316791	0.054866	(1)

TABLE XVII

Repletion of noradrenaline and dopamine and influence of distary sodium in the rat brain after a single injection of reserpine.

Table of Means.

Diet	Days after treatment 6	12	6	12
	noradrenaline		dopamine	
N.B.C.sod. def.	0.233 S.E.± 0.024 n:6	0.414 S.E.±0.021 n:8	0.328 S.E.±0.024 n:6	0.445 S.E.±0.021 n:8
N.B.C.sod. def. +Na	0.304 S.E.±0.024 n:6	0.440 S.E.±0.021 n:8	0.327 S.E.±0.024 n:6	0.477 S.E.±0.021 n:8

Values are in microgram/total organ
N.B.C. sodium deficient diet 1% NaCl in the food, controls had 1% corn starch
glass-distilled water for drinking
Reserpine 10 mg/kg body-weight s.c.

Analysis of variance.

Noradrenaline

Sources of variation	DF	S of F	MS.	F
Total (28)	27	0.209656	-	-
Days	1	0.119474	0.119474	33.47
Sodium	1	0.004521	0.004521	1.27
Interaction	1	0.000006	0.000006	1
Remainder	24	0.085655	0.003569	(1)

Dopamine

Sources of variation	DF	S of F	MS.	F
Total (28)	27	0.256707	-	-
Days	1	0.171359	0.171359	50.61
Sodium	1	0.002754	0.002754	1
Interaction	1	0.001325	0.001325	1
Remainder	24	0.081269	0.003386	(1)

and still persisted on the 12th day. In neither experiment could any significant interaction be established for treatments.

D. The Effect of Reserpine on Adrenalectomized Rats

Adrenalectomized rats and sham operated controls were divided by random procedure into treatment groups. One half of the animals of each group received reserpine injection, the other half, "placebo". The dose of reserpine in successive experiments was 0.1 mg/kg. - 0.2 mg/kg.

The adrenalectomized rats, maintained on a regular diet + 1% saline to drink, died without any exception within 5 - 7 hours after a single minimal dose of 0.1 mg/kg. reserpine in contrast to the adrenalectomized "placebo" injected and sham operated groups. The observed pre-mortem signs were the same as described before for animals dying in adrenal insufficiency. Adrenalectomized rats, pre-treated with 1 mg/100 gr. BW. desoxycorticosterone acetate subcutaneously before administering reserpine did not show these features even if a 1 mg/kg. dose of reserpine was injected. Apparently, the DCA protected the adrenalectomized animals from the lethal effect of reserpine.

E. Effect of Reserpine on Adrenalectomized - DCA Treated Rats

Adrenalectomized and sham operated rats received 1 mg/kg. BW. DCA daily for four days. Another adrenalectomized and sham operated group received 1 mg/100 gr. BW. hydrocortison in place of DCA, similarly for four day, starting immediately

after the operation. Reserpine, in a dose of 0.1 mg/kg. and 0.2 mg/kg. BW. respectively, was administered to the treatment groups, as indicated on Table XVIII, on the 4th post-operative day. At this time, it became obvious that hydrocortisone, like DCA, is capable of preventing the death of the adrenalectomized reserpine treated rats. The animals were sacrificed on the 12th post-operative day and their hearts and brains removed for estimating catecholamines.

Table XVIII shows the estimated values. It would appear from these that reserpine or adrenalectomy caused a drop in the level of adrenaline and noradrenaline in the heart, reserpine adrenalectomy caused no further drop. However, having established in the previous experiments that the repletion of catecholamines is very rapid and that there is a significant increase from the 6th day on if intact animals were injected with reserpine, we do not see any tendency for repletion in this adrenalectomized group. A very low level existed after 8 days of reserpine administration. We must, therefore, suppose a possible interaction of these treatments, assuming that one alters the other's action. This could be more clearly seen when studying the effect of these treatments on the catecholamines of the brain.

Table XIX summarizes the estimated values for brain catecholamines and Tables XX and XXI show the mean values together with their statistical analysis. From Table XIX, it appears that under these experimental conditions, the main effect of

TABLE XVIII

Heart

Treatment	0.1 mg. Reserpine + DOC Adrenaline	Noradrenaline	0.2 mg. Reserpine + Hydrocortone Adrenaline	Noradrenaline
Ax-Reserpine	(4) 0.022	0.05	(5) 0.002	0.04
Ax-Placebo	(6) 0.002	0.05	(5) 0.002	0.05
Sham-Reserpine	(10) 0.003	0.05	(8) 0.002	0.04
Sham-Placebo	(10) 0.036	0.29	(8) 0.040	0.38

Brain

Treatment	0.1 mg. Reserpine + DOC Noradrenaline	Dopamine	0.2 mg. Reserpine + Hydrocortone Noradrenaline	Dopamine
Ax-Reserpine	(4) 0.070	0.360	(5) 0.062	0.421
Ax-Placebo	(6) 0.239	0.370	(5) 0.229	0.427
Sham-Reserpine	(10) 0.192	0.420	(8) 0.192	0.421
Sham-Placebo	(10) 0.509	0.554	(8) 0.604	0.472

Mean values expressed in microgram/total organ.

Number in brackets show the number of animals used.

TABLE XIX

Brain

(Values are not true values, but summation of the previous table)

Treatment	0.1 mg. Reserpine + DOC		0.2 mg. Reserpine + Hydrocortone	
	Noradrenaline	Dopamine	Noradrenaline	Dopamine
Ax	0.309	0.730	0.291	0.894
Sham	0.701	0.974	0.796	0.893
Reserpine	0.26	0.78	0.25	0.84
Placebo	0.75	0.92	0.83	0.90

TABLE XX

Means.

Effect of reserpine-adrenalectomy on brain dopamine.

Treatment		0.1mg/kg reserpine + 1mg DOC	0.2mg/kg reserpine + 1mg hydrocortison
	reserpine	0.420 S.E.±0.045 n.10	0.421 S.E.±0.050 n.8
S.O.			
	placebo	0.554 S.E.±0.045 n.10	0.472 S.E.±0.050 n.8
	reserpine	0.360 S.E.±0.072 n.4	0.421 S.E.±0.064 n.5
Ax.			
	placebo	0.370 S.E.±0.059 n.6	0.427 S.E.±0.064 n.5

mean values are in microgram/total organ

S.O. sham operated

Ax. adrenalectomized

Analysis of variance

Sources of variation	DF.	S of S	MS.	F.
Total (56)	55	0.258697	-	-
Experiments (2)	1	0.000813	0.000813	1
Effect of Ax.	1	0.067584	0.067584	3.27
Effect of reserpine	1	0.051775	0.051775	2.51
Interaction	1	0.033197	0.033197	1.61
Remainder	51	0.105328	0.020652	(1)

TABLE XXI

Means.

Effect of reserpine-adrenalectomy on brain noradrenaline.

Treatment		0.1mg/kg reserpine + 1mg DOC.	0.2mg/kg reserpine + 1mg hydrocortison
S.O.	reserpine	0.192 S.E.± 0.012 n.10	0.192 S.E.± 0.014 n.8
	placebo	0.509 S.E.± 0.012 n.10	0.604 S.E.± 0.014 n.8
Ax.	reserpine	0.070 S.E.± 0.019 n.4	0.062 0.017 n.5
	placebo	0.239 S.E.± 0.015 n.6	0.229 0.017 n.5

mean values are in microgram/total organ

S.O. sham operated

Ax. adrenalectomized

Analysis of variance.

Sources of variation	DF.	S of S	M.S.	F.
Total (56)	55	1.964672	-	-
Experiments (2)	1	0.001413	0.001413	1
Effect of Ax.	1	0.584199	0.584199	38.94
Effect of reserpine	1	1.109633	1.109633	739.75
Interaction	1	0.192939	0.192939	12.86
Remainder	51	0.076488	0.001500	1

adrenalectomy is on the brain noradrenaline, little on the dopamine. The main effect of reserpine is also on the brain noradrenaline, little on the dopamine. Both the effect of adrenalectomy and reserpine proved to be statistically significant for noradrenaline as well as dopamine. The statistical analysis reveals also a significant interaction between treatments. In the previous experiments, it was demonstrated that adrenalectomy alone did not cause any significant change in the brain catecholamines, but in the presence of administered reserpine and steroids, the effect of adrenalectomy on the brain catecholamines, was to cause a decrease in these values which became statistically significant.

F. Urinary Excretion of Catecholamines in Adrenalectomized Rats

Adrenalectomized and sham operated rats were placed in metabolism cages and urine collected for 24 hours on alternate days. While in the metabolism cages, the animals received only tap-water but no food. During the alternate 24 hours, they were maintained in their cages on Purina Fox Chow and tap-water ad libitum. The first urine collection was made in the immediate post-operative 24 hours. The animals received post-operatively and thereafter daily, 0.5 mg/100 gr. BW. DCA subcutaneously.

As shown on Tables XXII, XXIII, XXIV, the urinary excretion of free catecholamines, adrenaline, noradrenaline, dopamine did not alter in adrenalectomized animals as compared to the sham operated controls.

TABLE XXII

Adrenaline

Treatment	body-weight		Days after operation. 1	3	5	7	9	11	14
	112		0.96	0.88	1.09	0.58	0.57	0.67	0.80
	110	urine vol./ml/24 h.	22	18	27	16	15	16	10
	98	n:	3	3	3	2	2	2	2
	103		1.17	1.53	0.89	0.87	0.87	0.88	0.85
Adrenalectomy	106	urine vol./ml/24 h.	18	29	16	15	17	24	25
----------	104	n:	3	3	2	2	2	2	2
	105	mean	0.36	0.40	0.39	0.36	0.36	0.39	0.41
	110		1.21	1.18	0.97	0.97	0.88	0.82	0.97
	97	urine vol./ml/24 h.	34	37	42	28	21	23	47
	118	n:	3	3	3	3	2	2	2
Sham operated	104		0.78	1.07	1.32	1.38	1.94	0.67	0.94
----------	103	urine vol./ml/24 h.	47	50	48	31	23	36	22
	107	n:	3	3	3	3	2	2	2
	106	mean	0.34	0.38	0.39	0.39	0.45	0.37	0.48

values are in g/24 hours urine n. animals
n: number of animals in 1 cage.

Table of Means.

Treatment.	Days after operation. 1	3	5	7	9	11	14
Adrenalectomy	3.43	3.81	3.71	3.43	3.43	3.71	3.90

Sx^2 92.5490
Sx 25.42
$\bar{x}$ 3.63
n 7
SE± 0.075

Table of Means. cont...XXII.

	Days after operation. 1	3	5	7	9	11	14	
Sham-operated	3.21	3.58	3.68	3.68	4.24	3.50	4.53	Sx^2 100.9538
values are in g/kilogram body-weight/24 hours urine								Sx 26.42
								$\bar{x}$ 3.77
								n 7
SE. of difference ± 0.188								SE± 0.171

P: <0.05 N.S.

TABLE XXIII

Noradrenaline.

Treatment	Body-weight		Days after operation. 1	3	5	7	9	11	14
	112		3.17	3.61	3.21	2.34	1.99	1.95	2.17
	110	urine vol/ml/24 h.	22	18	27	16	15	16	10
	98	n:	3	3	3	2	2	2	2
	103		2.92	2.73	2.37	2.46	2.47	2.29	2.53
Adrenalectomy	106	urine vol./ml/24 h.	18	29	16	15	17	24	25
----------	104	n:	3	3	2	2	2	2	2
	105	mean	1.01	1.06	1.11	1.20	1.11	1.06	1.17
	110		3.27	2.94	3.43	4.09	2.45	3.40	3.03
	97	urine vol./ml/24 h	34	37	42	28	21	23	47
	118	n:	3	3	3	3	2	2	2
	104		2.84	3.17	3.87	3.52	1.64	2.65	3.13
Sham-operated	103	urine vol./ml/24 h.	47	50	48	31	23	36	22
----------	107	n:							
	106	mean	0.96	1.01	1.22	1.27	1.02	1.51	1.54

values are in g/24 hours urine n. animals
n: number of animals in 1 cage

Table of Means.

Treatment	Days after operation. 1	3	5	7	9	11	14
Adrenalectomy	9.53	10.09	10.57	21.43	10.57	10.09	11.14

$S\bar{x}^2$ 772.6314
Sx 73.42
n 7
$\bar{x}$ 10.4885
SE± 0.246

Table of Means. cont...XXIII.

	Days after operation.						
	1	3	5	7	9	11	14
Sham-operated	9.06	9.53	11.51	11.98	9.62	14.24	14.53

values are in g/kilogram body-weight/24 hours urine

SE. of difference
± 0.745
P: <0.05 N.S.

$S\bar{x}^2$ 955.3479
Sx 80.47
n 7
$\bar{x}$ 11.4957
SE± 0.702

TABLE XXIV

Dopamine

Treatment	body-weight		Days after operation. 1	3	5	7	9	11	14	
	112		8.33	6.12	8.92	3.75	5.54	6.23	4.82	
	110	urine vol./ml/24 h.	22	18	27	16	15	16	10	
	98	n:	3	3	3	2	2	2	2	
	103		5.07	7.84	6.75	5.55	3.84	4.55	5.01	
Adrenalectomy	106	urine vol./ml/24 h.	18	29	16	15	17	24	25	
	104	n:	3	3	2	2	2	2	2	
	105	mean/1 animal	2.23	2.33	3.13	2.32	2.34	2.72	2.45	
			8,78	6.73	7.19	6.15	4.10	5.10	5.40	
	110									
	97	urine vol./ml/24 h.	34	37	42	28	21	23	47	
	118	n:	3	3	3	3	2	2	2	
	104		9.41	8.56	6.84	7.88	5.40	4.70	5.05	
Sham-operated	103	urine vol./ml/24 h.	47	50	48	31	23	36	22	Sx^2 4031.6352
----------	107	n:	3	3	3	3	2	2	2	Sx 166.84
										n 7
										$\bar{x}$ 23.8343
	106	mean/1 animal	3.03	2.54	2.34	2.33	2.37	2.45	2.61	SE± 1.15

values are in g/24 hours urine n. animals
n: number of animals in 1 cage

Table of Means.

Treatment	Days after operation. 1	3	5	7	9	11	14	
Adrenalectomy	21.24	22.19	29.81	22.09	22.28	25.90	23.33	Sx^2 4000.4106
								Sx 166.66
								n 7
								$\bar{x}$ 23.8086
								SE± 0.86

Table of Means. cont. XXIV.

	Days after operation. 1	3	5	7	9	11	14
Sham-operated	28.58	23.96	22.07	21.98	22.36	23.11	24.62

values are in g/kilogram body-weight/24 hours urine

S.E. of difference
± 1.44
P: <0.05 N.S.

The statistical calculation was made on the basis of g/kg. body-weight/mean of one animal's excreta over 14 days of experiment (7 days collection).

V. DISCUSSION

A. Effect of Adrenalectomy, Dietary Sodium with or without Administered Cortical Hormones on the Catecholamine Content of the Rat Heart and Brain

The original hypothesis of Banting and Hall (205) which postulated that organic effects of physiological dysfunction occur in one or more local areas as a result of an autonomic imbalance produced by persistent over-action of the parasympathetic has received support but profound criticism also from several other workers.

Hall and Cleghorn reported (2) that if dogs are allowed to go into adrenal insufficiency by withholding cortical extract, the electrocardiogram showed changing ventricular and auricular complexes. The first evidence of cardiac disturbance was the change to an irregular heart beat which was almost always followed by a sudden bradycardia. They suggested that this bradycardia is, at least, partly a result of the vagal preponderance and would indicate that at this stage of adrenal insufficiency, the mediator substance was either depleted or ineffective. Their further suggestion that this exhaustion or ineffectiveness of the mediator substance is not complete nor permanent is indicated

by the fact that even in the presence of severe insufficiency with prostration, where the electrocardiogram shows evidence of marked myocardial ischemia with auricular fibrillation or heart block, the heart may be restored to a normal rhythm by the intravenous injection of hypertonic glucose and saline, or by the injection of adrenal cortical extract or both. Our experimental results give a substantial support to this previously advanced hypothesis of Hall and Cleghorn.

It is apparent from our experiment that the level of estimated noradrenaline in the heart decreases progressively in the adrenalectomized animals if given but one injection of DCA immediately post-operatively, or none at all. A persistent low level was seen, if animals were maintained on daily injection of DCA, but no significant further decrease was noted with time.

Sham operated animals showed a similar pattern but a higher catecholamine level. The change in catecholamine content was not statistically significant, except in the group, where the animals received a single injection of DCA; then the decrease with time was significant. It is also seen from the experiments that despite increasingly or persistently low levels, a complete depletion of this catecholamine did not occur, though numerous animals were sacrificed in terminal adrenal insufficiency at 7 - 9 days post-operatively during the experiments.

Apparently, the noradrenaline values in the heart can not be evaluated in the sense of a stagnant, static view, but there are true values of a momentous happening of a dynamic

integrated or disintegrated action in a given condition. Studies on the failing heart (206) showed, using the isolated heart-lung preparation, that the presence of noradrenaline in the isolated heart does not prevent the development of spontaneous failure. This becomes particularly clear when the concentration of the norepinephrine in the heart muscle is increased by the addition of dopa to the perfusion fluid as shown by Von Euler (207). After the positive inotropic effect of the conversion products of dopa (dopamine, noradrenaline, adrenaline) has disappeared, the performance curve of the heart again shows the presence of failure though the concentration of noradrenaline in the heart muscle remains elevated. It is very likely that noradrenaline as present in the tissues is bound and in "storage form" without producing physiologic or pharmacologic effect. Apparently, "stored noradrenaline" does not affect myocardial efficiency and contractility, and only the nerve impulse can establish the link between the amine and the effector organ. The probable nature of this is that when the ester is freed, it is redistributed and acts upon the receptors, presumably a protein, and this action is responsible for the change in ionic permeability and the generation of bioelectrical phenomena.

The major portion of the heart catecholamines is produced at the sympathetic nerve endings within the heart. Cleghorn and Fowler (1) reported an apparent failure of sympathetic nerve stimulation and pressor drugs to produce the usual responses after

adrenalectomy. Accordingly, Woo Choo Lee and Shideman (208) found that in cats bilateral sympathectomy or administration of reserpine results in a marked reduction in the concentration of myocardial catecholamines and the contractility of papillary muscles from such animals were significantly less than that of muscles from untreated animals. 120 cases of heart disease, whether taken as a group or divided into disease entities, showed a preponderance of values well within or below "normal" range (209). The rate of local degradation and excretion of catecholamines is virtually unknown at present. Thus, the metabolism of catecholamines could be locally increased despite a low tissue level. The demonstration of increased urinary catecholamine excretion in hypertension and myocardial infarction suggests an increased catecholamine metabolism in these conditions (210).

Adrenolytic drugs are known to inactivate catecholamines that circulate in the blood, but not those that are stored within the myocardium. The contrasting electrocardiogram suggest an important contributory role of the extra circulating catecholamines in the origin of electrocardiographic abnormalities in individuals with hypertensive heart disease (211). Death can be caused by the injection of a massive dose of catecholamines, but their significance in unexplained cases of sudden death seems questionable (212). Bloodworth (209) assayed 120 cases by different methods and failed to uncover a single case of sudden death which could be attributed to excessive myocardial catecholamine concentration.

Probably the circulating level of catecholamines never reaches the concentration needed to cause death except perhaps in the cases of pheochromocytoma. However, in cases of pheochromocytoma, the level of circulating catecholamines may be quite high for years without causing death (213).

The relative independence of changes in the "left ventricular strain pattern" on the electrocardiogram from the blood pressure levels indicate that they are largely biochemical, biophysical and not merely mechanical in origin. According to Raab (214) the cardiovascular situation (diastolic hypertension) is practically identical in three otherwise heterogenous syndromes, namely, a. pheochromocytoma

b. hyperadrenocorticalism

c. essential hypertension.

This suggests the probable existence of a common pathogenic denominator. The hypertension in the first two syndromes is curable by removal of a catecholamine-producing pheochromocytoma or a corticoid-producing adrenal cortical tumor respectively. Essential hypertension may be ameliorated or cured by:

a. sympathectomy

b. adrenalectomy

c. sodium withdrawal.

Each of these methods attacks a fundamental mechanism of hypertension at a different point:

a. sympathectomy is in essence equivalent to the removal of a pheochromocytoma in that it reduces the amount of catecholamine-producing tissue,

b. adrenalectomy is equivalent to removal of an adrenal cortical tumor in that it eliminates the corticoid-producing tissue,

c. sodium withdrawal is comparable to adrenalectomy in that it deprives the body of material by means of which the mineralocorticoids exert their effects on the cardiovascular system.

The most important feature of DCA action consists of its unique ability to increase the intracellular concentration of sodium (215), thus to affect the intra-extra cellular electrolyte gradient. The latter has been shown to influence the electrical cell membrane potential which in turn determines the contraction amplitude of the individual muscle cell under adequate stimulation, in proportion to the magnitude of the intra-extra cellular electrolyte concentration difference (216). Sympathogenic catecholamines are known to act as re and depolarizing agents and that the cardiovascular contractile cells are constantly exposed to an influx of catecholamines, either by way of direct neurosecretion from the postganglionic sympathetic fibers or via blood stream from the adrenal medulla.

According to Raab (214) the blood pressure level appears to be influenced by the integrated interaction of:

a. the quantity of catecholamines in the heart and vascular walls,

b. the quantity of sodium within the cardiovascular cells (intra-extra cellular electrolyte concentration gradient),

c. the quantity of the sodium depositing mineralocorticoids as regulators of b. and thereby of the effects exerted by a.

Several approaches have been attempted as to what are the neurophysiologic effects of the sympathomimetic amines. Marazzi (217) found, in his survey of a variety of sites in the nervous system, that consistent reciprocal relationship existed between excitation or enhancement by acetylcholine and acetylcholine-like substances on synaptic transmission phenomena, and inhibition by adrenaline, noradrenaline and other sympathomimetic amines. He believes, on the basis of his experiments, that there exists an equilibrium of neurohumoral control of transmission in cerebral synapses and through the nervous system. This is susceptible to distortion and imbalance by disturbance in the amount of chemical regulator or the susceptibility of the receptors. Eccles (218) demonstrated that there is an interplay between excitatory and inhibitory effects. Accordingly, an impulse reaching an excitatory synapse operates by liberating a transmitter substance which causes a brief ionic current of flow inward across the subsynaptic membrane. Correspondingly, the inhibitory impulse liberates a transmitter which causes the subsynaptic membrane to generate a current that tends to hyperpolarize the whole postsynaptic membrane. Adrenaline, noradrenaline have already been mentioned as inhibitory substances, mescaline and LSD behave similarly in this respect. According to Kuffler (219) gamma -amino-butyric acid (GABA) may activate the inhibitory mechanism

directly.

Our results permit a modification of Raab's concept. that is, that the quantity of noradrenaline in the heart is influenced by the amount of DCA or any mineralocorticoids present. However, failure of the heart may occur in the presence of measurable catecholamine concentrations, so one must look for disturbances not connected with extraction of the substances or the utilization of its metabolites. It would seem that the spontaneous failure of the heart results from an inability of the heart to utilize its catecholamine stores and in some disturbance in the mechanism connected with energy production. The decreasing ability of the heart to utilize its catecholamine stores is due to the fact that the effector organ is being deprived of an "active principle", in our experiments, DCA, which would act upon the receptor, change the ionic permeability of the cells, generate bioelectrical phenomena, which is in fact the "nerve impulse" which establishes the link between the amine and the effector organ. The progressively diminishing concentrations in the noradrenaline of the heart is probably a representation of a balance between its rate of release into the heart. At 7 - 9 days, post-operatively, the values for adrenalectomized animals probably represent the concentration of the "stored" or "inactive" noradrenaline, which cannot be activated by sympathetic discharge any more.

The elevation of the values for the sham operated animals in the immediate post-operative 24 hours can be accounted for by a stress induced catecholamine accumulation in the myocardium,

described by Raab (220) and Selye (221).

The evidence for differential involvement of organs and tissues in the course of shock and their significance together with the effects of such procedures as conditioning and the various types of treatment on the metabolic and circulatory changes has been evaluated by Engel (222). We can divide the sequence of events in response to haemorrhage or trauma leading to shock into three phases. The first, the initial response to the injury, involving a number of metabolic changes which are non-specific in the sense that they are independent of the type of injury. The next, the intermediate stage, between the initial response and the late stage when fairly characteristic changes in the metabolism and circulation take place. Somewhere in here something happens which converts this condition from one which readily responds to blood and plasma to one which goes on to death despite fluid replacement, the so-called irreversible shock. It has long been suspected that the adrenal cortex is involved in some way in shock. We shared the experience of others, that adrenalectomized animals are extraordinarily sensitive to any traumatic procedure and will readily go into circulatory collapse and death.

It is also known that individuals who may have apparently normal endocrine function but who are malnourished, chronically ill or depleted in protein in some way are somewhat more prone to develop shock when injured than are normal individuals.

Ingle (223) studied nitrogen excretion in normal and adrenalectomized animals after leg fracture and demonstrated that in normal animals, there is an increase in nitrogen excretion, while in adrenalectomized animals which were maintained on saline, there was no such change. When the adrenalectomized animals were maintained on a constant dose of cortical hormone and the same injury induced, there was exactly the same response in nitrogen excretion in the adrenalectomized animals as in the normal animals. In later work, (224) Ingle has shown the same response with respect to sodium chloride retention and a number of other phenomena. He suggested that a continued increase in adrenal hormone is necessary to sustain this response. Engel (225) showed that adrenaline stimulated a change in nitrogen metabolism of normal animals in three hours. When the same dose of adrenaline was given the adrenalectomized animals, there was no change in the nitrogen metabolism. When adrenaline was injected into adrenalectomized animals maintained on a crystalline suspension of cortisone in saline, there was a prompt increase in nitrogen metabolism lasting for three hours, just as in the normal animal. These data indicate that the adrenal cortex intervenes in metabolic responses to injury in some manner. However, it is possible, as reported by Baez (226) by careful training to get an animal that has been adrenalectomized and maintained on salt without adrenal extracts to withstand a degree of shock which is usually fatal for even a normal animal. The

development of resistance to trauma by this non-hormonally-conditioned manner is not accompanied, however, by resistance to the effects of adrenal insufficiency per se.

A clue to the possible mechanism of spontaneous failure of the heart may lie in the work of Engel (225) who showed that the inclusion of liver and spleen in the perfusion circuit of a heart-lung preparation increased myocardial efficiency. The possible pattern in animals that are going on to progressive failure or sudden circulatory collapse is the forced reduction in blood flow through the liver and the interference with hepatic enzyme systems. Experimental data (225) indicate definite abnormalities in protein metabolism due to slowing of circulation in the liver and due to hepatic anoxia but at present, we cannot contribute these changes to the sudden circulatory collapse. Carlsson (227) presented a spectrum of changes in the various carbohydrate intermediaries, adenosine triphosphate, adenosine diphosphate, and other phosphate stores which showed a very marked depletion in all kinds of shock. Gerola et al. (228) data suggest that the decline in systolic force of the papillary muscle, under several conditions affecting the force of their contraction, is due to the fall in concentration of ATP, which is the result of the deficiency of the total adenosine nucleotide. Knowing that the catecholamines are coupled with ATP and that of the "catecholamine granules" are the highest source of ATP, the decrease in catecholamine content of the heart with the consecutive cardiac failure observed in our experiments can be

explained on a possible ground of total adenosine nucleotide deficiency.

BRAIN

The presence and distribution of noradrenaline, dopamine and questionable adrenaline and dopa in the brain has been demonstrated by several workers. Noradrenaline appears to be detectable in all parts of the brain, though in varying concentrations. The highest amounts are found in the diencephalon, and the medulla oblongata, where very little dopamine can be detected. On the other hand, the sites with the highest dopamine content contain little noradrenaline. A study of the distribution of dopamine in the brain has shown that the amine is predominantly localized in the corpus striatum of the hemispheres. In amphibia, it was found that dopamine contributes very little to the total catecholamine content of the brain. In them, the principal catecholamine in both the brain and the peripheral organs, is adrenaline. The distribution of dopamine in the brain and other tissues indicates that it may have a function of its own. Probably dopamine is concerned with the function of the corpus striatum and thus, with the control of motor function. Drugs which influence the dopamine content of the corpus striatum also produce disturbances in motor activity. Excess of dopamine in the brain produced by administration of dopa is accompanied by motor hyperactivity (229).

In our experiments, we were not able to obtain clear-cut

evidence of a possible influence of endocrine function on the brain catecholamines. The concentration of noradrenaline and dopamine in the brain did not alter after the animals were subjected to adrenalectomy. De Meio (230) reported differential increases of serotonin concentration in pooled fractions of the base, hemispheres and medulla oblongata of the rat brain 24 hours after bilateral adrenalectomy or hypophysectomy. Their work was repeated by Towne and Sherman (231) using chemical fluorometric procedure for estimating serotonin. Their experiments showed no effect on the brain serotonin level as a result of acute bilateral adrenalectomy. One of our experiments showed, however, a differential increase in dopamine of the brain, after adrenalectomy. These animals exhibited extreme irritability and motor hyperactivity. However, this finding could not be confirmed in the consecutively repeated experiments.

B. <u>The Effect of Reserpine on Adrenalectomized Rats</u>

The depleting effect of reserpine on body stores of 5-hydroxytryptamine and the catecholamines is now well established. In reserpinized animals, the peripheral part of the adrenergic system does not function owing to lack of the transmitter. This is also true for the central part of the adrenergic system, and it is probably responsible for the observed tranquilizing effect. Administering the amino-acid precursor 3, 4 dihydroxyphenylalanine which has been shown to penetrate the blood - brain barrier readily, is followed by an increase in the level of catecholamines

in the brain as well as by central excitation (229). If animals are given iproniazid about 2 hours before the 3, 4 dihydroxy-phenylalanine, the dose of the latter required to antagonize the effect of reserpine is markedly reduced (232). This supports the assumption that the effect of administered precursor was due to an amine formed from it. Catecholamine concentrations in the brain appear to be influenced to a large extent by the amine oxidase inhibitor, iproniazid. Since iproniazid inhibits monoamine oxidase, it results in an increase in serotonin and catecholamine concentration in the central nervous system. Thus, reserpine leads to a diminution of serotonin while iproniazid leads to an increase, as it does the catecholamines.

Noradrenaline is concentrated in those portions of the brain which, when stimulated, produce a pressor response (233). In addition to a pressor effect, it is possible to elicit from the hypothalamus practically all responses mediated by the peripheral sympathetic nerves. A high concentration of serotonin has also been reported in the central nervous system, particularly in the diencephalon. These regions are of great importance in the automatic regulation of vegetative processes. There appears to be a parallelism between the concentration of noradrenaline and those of serotonin. This finding points to a correlation between the two amines. Bing (306) found that insulin caused a depression of the noradrenaline content of the hypothalamus, and ether anesthesia reduced that of area postrema. It appeared

that substances which cause a secretion of catecholamines by the adrenal medulla are particularly active in depleting the brain of noradrenaline. It is established (214) that reserpine therapy depletes the patient's heart and certain parts of the brain of catecholamines. This could conceivably lead to cardiovascular embarrassment and might explain the sudden fall in arterial pressure and death in certain patients under reserpine therapy when they undergo anesthesia or electroshock therapy.

Our experiments showed that adrenalectomized rats maintained on a normal diet died within hours after an injection of 0.1 mg/kg. reserpine. Adrenalectomized rats receiving 1 mg/100 gr. of DCA daily did not show this feature after reserpine. These findings are in agreement with observations of Ashwin (234) which showed that when death occurred, the animals exhibited diarrhoea and intraintestinal haemorrhage. Sham operated animals showed only mild diarrhoea. It was concluded that death results from the loss of fluid and blood from the intestinal tract which has been deprived of the stabilizing benefits of adrenal hormones and then stimulated by a sudden release of mucosal serotonin. DCA is believed to protect these animals by its ability to control the loss of water and electrolytes. In our experiments, it was shown that the adrenalectomized-reserpine treated animals maintained on DCA have their noradrenaline and dopamine content of the brain depleted. Both reserpine and adrenalectomy were shown

to have a significant role in this result. There is also a significant interaction of the treatment. The meaning and significance of these findings are inexplicable at present.

C. Urinary Excretion of Free Catecholamines Following Adrenalectomy

It was shown by Holtz and workers in 1947 that a certain amount of sympathomimetic amines is normally present in the urine. He inferred from his experiments that these amines were adrenaline, noradrenaline and dopamine, which was confirmed by several other investigators. The adrenaline output in the urine when the adrenals have been removed in patients fall to zero, according to Von Euler (236) et al. but the noradrenaline output did not change to any great degree. They suggested the explanation that the source of adrenaline output is chiefly the adrenals and the noradrenaline comes from other sources, presumably the adrenergic nerves. However, he and his co-workers could not confirm this finding later and their subsequent report showed no change in excretion of adrenaline and noradrenaline after adrenalectomy.

Luft and Euler (237) found that in postural hypotension which has a direct relationship to the shock problem, the output of adrenaline and noradrenaline was greatly reduced. These patients did not respond to insulin neither with an increased adrenaline secretion. Apparently in this condition, the ability of the adrenergic nervous system to produce noradrenaline was

impaired to such an extent that they did not secrete or liberate enough of the substance. It is of particular interest to note Shorr's observation (238) that adrenalectomized salt-maintained animals, which were very susceptible to any kind of shock, a pre-treatment with Dibenzyline (phenoxybenzamine) resulted in 100% survival. Millar et al. (239) showed that phenoxybenzamine administration in dogs led to a marked increase in urinary adrenaline and noradrenaline excretion, together with an increase in the plasma adrenaline and noradrenaline concentration. There was no significant rise in plasma adrenaline concentration in adrenalectomized animals treated with phenoxybenzamine, but the noradrenaline showed a gradual increase after the drug administration. The increased concentrations of adrenaline and noradrenaline when circulatory collapse was imminent due to haemorrhage in the dogs treated with phenoxybenzamine was not significantly different from the terminal concentrations estimated in the untreated dogs. However, in the adrenalectomized dogs treated with phenoxybenzamine the terminal rise in arterial noradrenaline concentration was significantly greater than those observed in adrenalectomized animals not treated with phenoxybenzamine. In our experiments, the adrenalectomized cortical hormone maintained rats did not show any change in urinary adrenaline, noradrenaline or dopamine excretion as compared to the sham operated similarly maintained rats, during the 14 days of experimental period. This finding would suggest an extra-

adrenal source of adrenaline excretion. However, due to the uncertainty of the method for adrenaline, the obtained values are disputable.

VI. SUMMARY AND CONCLUSION

1. Evidence has been presented that, following adrenalectomy, there is a decrease in the noradrenaline content of the rat heart. The experimental results show a definite effect of the mineralocorticoid, desoxycorticosteron acetate, in maintaining a "normal" level of catecholamine, particularly noradrenaline in the heart. The possible mechanism of such an effect has been discussed and it was concluded that the effect of DCA upon the noradrenaline of the heart is not entirely due to its salt regulating activity, but to some other property. This view is supported experimentally by the fact that dietary sodium did not play a significant role upon these changes after adrenalectomy.
2. The sudden death of the adrenalectomized rat after reserpine treatment has been described. It was shown that DCA prevented death after reserpine in such animals. The preventive effect of DCA has been discussed.
3. Data has been presented showing decreases in the catecholamine content of the rat heart and brain following adrenalectomy and reserpine treatment, the animals being kept alive by

DCA treatment.

4. No changes were found in the catecholamine content of the brain following adrenalectomy, with or without administered steroids.
5. Evidence has been presented that the urinary excretion of adrenaline, noradrenaline and dopamine did not alter following adrenalectomy in rats.
6. Consideration of the data presented in this thesis contributes to a knowledge of the differential involvement of the organs to "stress" and suggests a probable cause of sudden, unexplained deaths which occur in men and several other species.

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