EXPERIMENTAL STUDIES OF THE EFFECTS OF CATECHOL AMINES ON GASTRIC SECRETION

by

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PREFACE

A tremendous expansion of the investigation of the adrenergic mechanisms can be observed in recent years. This is undoubtedly due to the fact that the importance of the catechol amines in many physiological functions is realized to an increasing extent, as well as to the pursuing of the studies of the basic nature of chemical transmission. One must indeed agree with the statement of Sir Henry Dale, the discoverer of the action of adrenaline, that it is heartening to see such a recognition and accelerated extension of studies in this fascinating subject.

This development coincided with the recent reactivation of the investigation of gastric secretion. To use the words of Dr. D.R. Webster, Director of the Department of Experimental Surgery, McGill University: "We have been living on the Pavlovian principles for many years and it is time that the older results were re-examined in the light of modern methods of research." The effects of Adrenaline and sympathetic nerve stimulation on gastric secretion have been studied at McGill by the late Professor B.P. Babkin and his co-workers, who have observed many principle facts of their action. Because of this tradition in our laboratory, as well as my personal interest, I have gladly followed the suggestion of Dr. Stanley C. Skoryna, Director of the Gastro-Intestinal

Research Laboratory at McGill University, to undertake the investigation of the effects of catechol amines on gastric secretion. The availability of many compounds, which affect both catechol amines and gastric secretion and which were not obtainable before, made this suggestion even more challenging. I wish to thank Dr. Skoryna sincerely for his constant encouragement, supervision and guidance in carrying out this project. Without this the work could not have been completed. In the initial period of studies many difficulties have been encountered, particularly with reference to establishment of a satisfactory method of estimation of catechol amine levels. During this period I was greatly helped by the suggestions of Dr. B. G. Benfey, Assistant Professor of Pharmacology, and Dr. Sourkes of the Research Laboratory of the Allan Memorial Institute. These difficulties were finally overcome with the advice from Dr. R. Hobkirk, Assistant Director of the Medical University Clinic of the Montreal General Hospital, who has kindly offered full co-operation in establishing of the use of catechol amine estimation in our laboratory. Mr. P. L. Rojowski, M. Sc., has then added some modification and carried out all catechol amine estimations, for which he is sincerely thanked, particularly in view of the great deal of time he spent in perfecting the methods used.

Dr. D. R. Webster, Professor of Surgery and Surgeon-in-Chief of the Royal Victoria Hospital, is thanked for offering the facilities of the Department of Experimental Surgery as well as those of the Surgical University Clinic at the Royal Victoria Hospital, during the investigation of clinical cases. Although the clinical results are not presented here, their initiation will be undoubtedly of value for future workers in this field.

Dr. Martin M. Hoffman, Consultant of the Laboratory, Dr. R. D. McKenna, Assistant Professor of Medicine, Dr. Schaffenburg of the Ciba Foundation and Dr. William T. Irvine of London, England, have offered generously their advice and help during discussions of the subject and research studies.

To Dr. F. C. McIntosh, Professor of Physiology, the author is greatly indebted for his stimulating teaching of physiology, which induced the author to study this subject in more detail than ever before. Dr. D. M. Edward was most helpful with numerous suggestions on the biochemical methods used.

The inestimable help of Miss Anne Watkins, Chief Biochemistry Technician, in carrying out accurately the estimation of gastric secretory components, and being most helpful in many other ways, is deeply appreciated.

Mr. Michael Farrell, Mr. James Byers and Mr. Serge Podymow have assisted the author in carrying out the numerous experimental surgical procedures (over 2,000 operations have been done) and care of the experimental animals. Miss Unni Mürer, Secretary of the Department, was most kind in carrying out of the correspondence on the subjectant ordering the laboratory supplies for the operation. She also contributed by her cheerful personality to the atmosphere of friendship in the Department, which made the ups and downs of research work look easy. It was a pleasure to spend these two years of study with Drs. Beaudry, Wekselman, Makhani, Duaky, Wright, McSweeney, Rodriguez, Makonnen and Mr. Pierre Morin.

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CHAPTER I

INTRODUCTION

The physiology of catechol amines has been subject to intensive investigations ever since it was recognized or more precisely postulated that differences exist in the nature and actions of Adrenaline and its demetylated precursor the Noradrenaline.

There are indeed few subjects in physiology which have been studied and given rise to more controversies. Part of the confusion which arose was probably due to different nomenclature of authors and compounds studied; part of it was undoubtedly due to the multiplicity of actions and variability of effects under different experimental conditions. Over forty years have passed before Noradrenaline, which was synthetized by Holz (1904) was demonstrated by U.S. von Euler, as the active substance of adrenergic nerve fibres, Sir Henry Dale and his co-worker George Barger were first to describe the action of sympathomimetic amines in 1910.

Numerous investigations have followed, but the great advances only began after 1946 - 1948, when U.S. von Euler demonstrated that Noradrenaline is the adrenergic transmitter. He first obtained definite

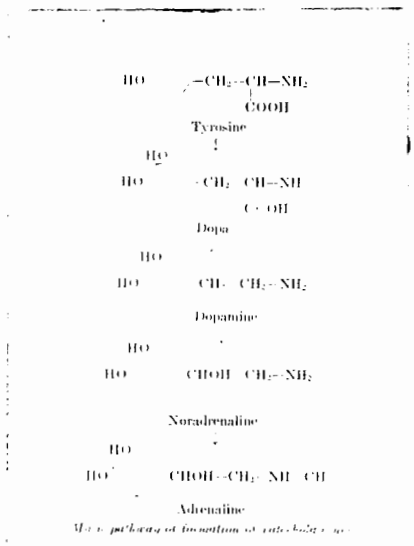
evidence of various actions of sympathicomimetic substances and this formed the basis for the rapid expansion of knowledge in this field.

The following thesis does not claim to be complete, with respect to the more recent findings. In many cases the evidence available is neither definite nor has it been verified by independent groups of investigators. The emphasis has been laid on the effects of metabolic products which also affect gastric secretion and therefore, are of particular interest in the studies of their relationship.

CHAPTER II

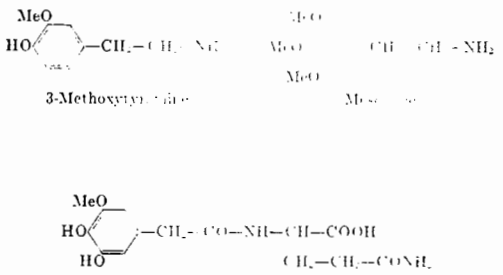
CHEMISTRY AND FORMATION OF CATECHOL AMINES

Both adrenaline and noradrenaline originate from the amino-acid Tyrosin. The following steps in the formation of catechol amines in man have been demonstrated by Blashko (1939).



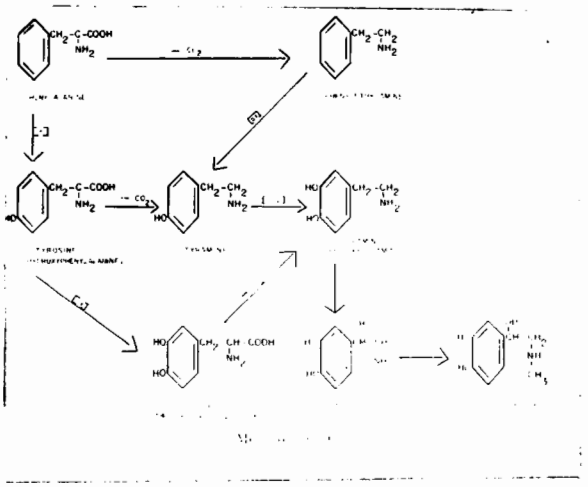
In some species such as the octopus the formation of catechol amines begins with Tyrosin, with the difference, that the second phenolic Hydrogen group is introduced. (Blaschko 1959).

Another possible mode of C.A. formation is by branching off from the main pathway. We have to consider the fact that Dopamine is also undergoing O - Methylation. Therefore the occurence of a compound closely related to Mescaline might be expected and its metabolism through partial demetylation, which is known to occur in the human body, results in the following compounds: (Clark 1959)



It has been proved recently (Clark,1959), that all the plasma oxydases act on Mescaline.

The latest findings concerning the formation of C.A. are described by Strauss and Wurm (1960). These authors claim that the two precursors of catechol amines are Phenylalamine and Tyrosine, both derived from dietary proteins.

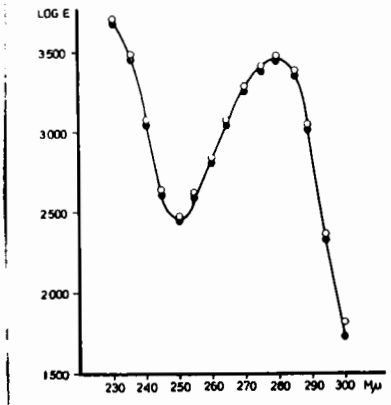


Through a series of enzymatic reactions, namely by decarboxylation, oxydation and deamination, which occur mostly in the liver and the kidneys, they are transformed to Dihydroxyphenylalamine,(Dopa) and Dihydroxyphenylethylamine(Dopamine).

The conversion to Noradrenaline takes place in the sympathetic nervous system, as well as in the adrenal medulla. Further

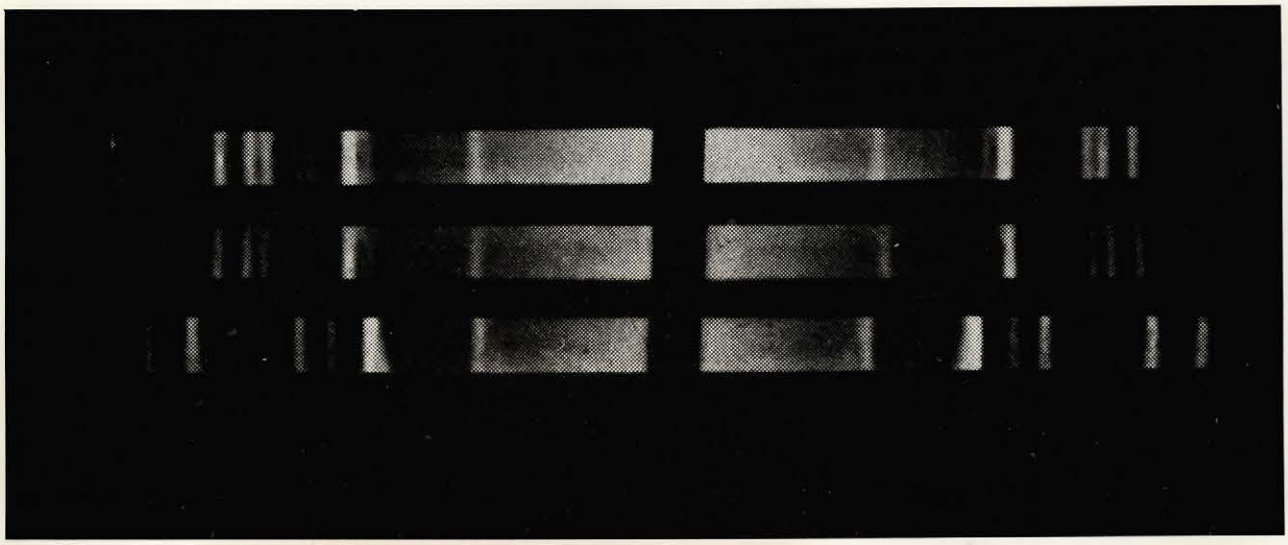
methylation which results in adrenaline formation, occurs only in the adrenal medulla and possibly in the extramedullary pheochrom system. Methyl donors for the latter process are mainly S-adenosylmethionine which also derive from alimentary sources. (Blaschko 1948).

U.S. von Euler and Hamberg (1950) have studied the ultraviolet absorption curve and the X-ray diffraction pattern of Noradrenaline. The absorption curves of synthetic and isolated Noradrenaline in water containing one equivalent of hydrochloric acid, was depicted by these investigators.



Absorption curves of synthetic (●) and isolated (○) noradrenaline in water containing one equivalent hydrochloric acid. (S. Bergström, U. S. v. Euler and U. Hamberg, *Acta physiol. scandinav.* 20:101, 1950.)

Absorption curve of synthetic (...) and isolated (ooo) Noradrenaline in water containing one equivalent hydrochloric acid.



X-Ray diffraction patterns of isolated (A) and synthetic (B) Noradrenaline. C is Adrenaline.

The colorimetric methods were studied by U.S.von Euler and Hamberg (1949) and are based on the formation of Noradrenochrome and Adrenochrome. Since Noradrenaline and Adrenaline occur in the animal tissues in a variable mixture, the adrenochrome formation is complete, when iodine is allowed to act for 90 seconds at pH 4.0. Only 10% of Noradrenaline is formed with the same tissue and under the same circumstances. A 3 minute treatment with iodine at pH 6.0 produces the maximal formations of Noradrenochrome and Adrenochrome.

The wave length 529 mu in a photometer indicates the presence of Noradrenochrome at pH 4.0. Adrenochrome data are similar on oxydation at pH 4.0 and pH 6.0 at the wave length of 529 mu. The molecular weight of Noradrenaline was found to be 169,18 as compared to Adrenaline 183,20 . Racemisation was studied by B.F.Tullar (1948) for Arterenol and by Kisbye and Schon (1948) for Noradrenaline. B.F.Tullar (1948) found that d - arterenol is almost quantitatively converted to its methylether by evaporation in vacuo; a solution of the hydrochloride in methanol suggested that ether formation occurs during resolution attempt in an hydrous alcohol.

Resolution of d-arterenol and aminoethyl 1 - 3, 4 di-hydroxy-benzyl-alcohol has been described recently, as well as characteristics of active l-isomers. Only l-art-erenol forms a hydrated salt with d-tartaric acid in aqueous alcohol solution:d-arterenol-d-tartrate.

D-arterenol-d-tartrate crystallized twice from aqueous solution and from 95% methanol is converted to the free bases by treatment with amin-hydroxide. The hydrochlorides were prepared by disolving the base in isopropanol with some HCl and crystallized by cooling.

CHAPTER III

STORAGE AND RELEASE OF CATECHOL AMINES

1. Storage

Adrenaline and Noradrenaline are the primary catechol amines of the blood. They can be found equally distributed in the blood corpuscles as well as in the plasma. They are also stored in the blood platelets. The concentration of catechol amines in the blood was controversial for a considerable period of time. At present it is generally agreed, that the mean content averages 1 mcrg. of combined catechol amines per liter of serum. The main site of storage of catechol amines is the adrenal medulla. Schumann found that the medullary granules, the organelles of 50 - 90 mu in diameter with a real membrane, contain the following constituents : 1.ATP (adenosine triphosphate). 2.catechol amines. 3. a protein. 4. water. Schumann and P. Hagen (1959) claim that there are two types of granula; lighter and heavier ones containing Adrenaline or Noradrenaline. Schumann and Hagen postulated, that Adenosine triphosphate was alway present with catechol amines. ATP and catechol amines were thought to represent the main weight of the dry adrenal (medullary) granula, found in

vivo in an inactive molar suspension neutralizing each others charges (Hillarp and Hagen 1959). The proportion to each other is about 4:1 (C.A. 4, ATP. 1) in humans and rats, 7:1 in chickens.

It is known, that the hydroxylation of Dopamine to Noradrenaline takes place in the large granule fraction of the adrenal medulla. It is not certain, whether the methylation of Noradrenaline to Adrenaline takes place inside or outside the granula or during the transport from one organelle to the other. Schumann could show in many experiments, that the granular membrane is permeable to ATP, catechol amines, water, potassium, chlorides and sucrose.

Fortier and Leduc (1957) claim that besides the four main constituents of the medullary granules, there were also Atipase and Succinicoxydase found, the function of which is not clarified.

Besides the adrenal medulla, Noradrenaline is stored in the sympathetic ganglia and the post ganglionic sympathetic nerve fibres. The distribution of catechol amines in various organs differ. The mucosa of the gastro-intestinal tract contains almost exclusively Dopamine, 99% of the total body content. This

seems to be related to the absorption of phenylalamine and tyrosine from the intestinal tract. Dopamine is a conversion product of these amines. To our knowledge the estimation of Noradrenaline and Adrenaline content of the gastro-intestinal wall has not been carried out up to the present time, although such studies might be of considerable importance.

The brain and the sympathetic nerve cells contain almost equal proportions of Noradrenaline and Dopamine . The lungs store high amounts of Dopamine as well as the liver and the kidneys. This would confirm the theory of transformation of the primary amines Tyrosine and Phenylalamine to Dopa and Dopamine mainly in the liver but also in the kidneys. It could be also proved by the fact that these two organs especially contain high amounts of Dopadecarboxylase, which is necessary for the decarboxylation of Dopa to Dopamine(Holtz 1959). Another specific localisation of Dopamine is the Splanchnic nerves, where it represents a half of the total catechol amines.

Apart from the function as a precursor of Noradrenaline and Adrenaline, the role of Dopamine is not well understood. One could postulate, that

the high amount of Dopamine stored in the intestinal tract could function there as a local hormone.

2. Release of Catechol Amines

The release of catechol amines is a complex process. It may be brought upon by various conditions whenever the metabolic needs of the tissues are increased. The physiological factors increasing the release of catechol amines are muscular exercise, the ingestion of certain foods such as bananas and thyroxin-containing materials. The well known Cannon reflex which cause high output of Noradrenaline into the system occurs frequently in every day life of animals and probably in humans too.

As pathological factors increasing catechol amine release can be enumerated the development of pheochromocytoma, stress, various mental disorders, Cushing's disease, thyroidtoxicosis, jaundice, lymphoma, hemolytic disease, renal disease, coronary thrombosis and administration of various drugs.

The main pathway of the release of catechol amines from adrenal medulla is mediated through stimulation of the splanchnic nerves. Central or reflex stimulation causing the liberation of catechol amines from the adrenal medulla are factors like stress, anoxia or sudden excitement.

This centrally located stimuli probably reach the

adrenal medulla and the sympathetic nerve fibres through the spinal cord. Insulin hypoglycemia also is known to cause release of C.A. from the adrenals through direct action on the hypothalamus. For instance electric stimulations of the hypothalamus or the cerebral cortex cause the release of C.A. into the adrenal venous blood.

Adrenergic neurones release probably mainly or exclusively Noradrenaline. The Dopamine present in sympathetic nerves is possibly liberated as well but there is no evidence available at present. Whether Dopamine serves only as precursor or as supply for Noradrenaline, or whether it has a function of its own is not known. Sympathetic nerve cells and the chromaffin cells of the adrenal medulla are embryologically derived from the same precursor cells, therefore the pharmacological effect of certain drugs on adrenal medulla as well as on the sympathetic nerves are the same.

Drugs like Acetyl choline, choline, nicotine, lobeline, potassium chloride, pilocarpine, tetramethylammonium, histamine, morphine,veratrum alkaloids and serotonin cause the release of C.A. from adrenal medulla and the sympathetic nerve fibres, On the other hand there are a few differences in action of cyanides and anoxia on sympathetic nerve fibres and the adrenal medulla.

Both anoxia and cyanides cause a catechol amine release from the adrenal medulla, but not from the sympathetic nerve fibres.

Antihistamines are also known to stimulate the release of C.A. from the adrenal medulla but at the same time they depress the excitability of sympathetic ganglion cells.

In the past years Reserpine has been found to be the most effective drug in depleation of C.A. from the adrenal medulla, the sympathetic nerves, the heart and the brain.

It seems that this disappearance of C.A. from the above mentioned organs is more likely caused by a release of the amines from chromaffin tissue, than by inhibition of synthesis.

The mechanism of release is not known. It may be so intensive , that it could interfere with the transmission of nerve impulse to the effector organs. In experiments carried out by Coupland (1959) transplantations into the anterior eye chamber of adrenal tissue previously depleted of C.A. by Reserpine, showed a lag period of 7 - 21 days until the normal levels of C.A.

were restored in the depleted adrenal tissue.

Some authors as J.A.Rider, H.C.Moeller, J.O.Gibb (1959) claim that the action of Reserpine is a direct one, causing depression on the hypothalamus. Latest results show that Reserpine has a biphasic action, central as well as peripheral, causing for instance an increase in gastric secretion which again is supposed to be in connection with the release of serotonin.

Nicotine, specificly causes, release of Noradrenaline from the adrenal medulla (P.Hagen 1959) without affecting Adrenaline release.

At present it is not possible to determine whether there is a selective release of Adrenaline or Noradrenaline, except a few drugs like Nicotine etc. on central stimulation, since the amount released are very small and the bio-assays are based on mixtures of the C.A. in adrenal venous blood. But there is evidence that the proportions of Adrenaline and Noradrenaline in the adrenal venous blood are the same as in the adrenals.

CHAPTER IV

METABOLISM OF CATECHOL AMINES

1. General Aspects

The metabolism of Epinephrine and Norepinephrine has been studied by numerous investigators. The principal data are available from the work of Armstrong, McMillan and Shaw (1957), and Axelrod (1957) in human subjects and in the rat. It has been shown that epinephrine and norepinephrine are metabolized by a combination of O-methylation and oxydative deamination. The following major metabolic components are produced: 3-methoxy-4-hydroxy mandelic acid (VMA), the 3-methoxy analogues of the amines like Metanephrine and Normetanephrine and 3,4-dihydroxy mandelic acid (DMA).

O-Methylation. Armstrong (1957) has found, that 3-methoxy-4-hydroxy-mandelic acid is an important constituent of human urine. The levels of this compound have been found to be markedly increased after infusion of norepinephrine as well as in patients with pheochromocytoma. In the latter group high levels of Normetanephrine have been also discovered, either in free or conjugated form. These observations appear to indicate, that O-methylation forms an important pathway in the metabolism of catechol amines in man and the rat.

Similar studies have been carried out using radioactive Epinephrine, (la Brosse, Axelrod, Kety 1958). These authors have found, that from 90% of the excreted radioactive E. in man,

55% was identified as metanephrine, 30% as VMA and less than 3% was 3,4-dihydroxymandelic acid (DMA).

In the rat Axelrod (1957), later Axelrod, Inscoe, Senok and Witkop (1958 a & b) found, that administration of tritium labeled E. results in appearance of high levels of synthetic metanephrine in the urine. Approximately 87% of the E. which was administered i.p. was recovered within 24 hours in the urine. The propertions of the metabolites of C.A. were as follows: metanephrine and normetanephrine: 55%, VMA:12%, DMA:traces only.

The main end products of O-methylation are metanephrine and normetanephrine. When radioactive metanephrine was administered to the rat, the same amount of metanephrine could be recovered in the urine whithin 24 hours in both free and conjugated forms. Metanephrine and normetanephrine were found physiologically in the rat urine and in certain tissues, (Axelrod 1957, Axelrod, Senok and Witkop 1958), like the spleen and the adrenal glands. In the urine there is more normetanephrine present than metanephrine. The adrenals contain about equal quantities of both, while the spleen shows only evidence of normetanephrine. The physiological activity of these metabolites are very weak in comparison with the unmethoxylated hormones epinephrine and norepinephrine, as shown by administration to man. This proves that O-methylation of C.A. is an inactivation process.

Deamination is the process in which the O-methylation metabolites are further transformed to their end products. In this transformation monoamine oxidase seems to be involved.

Many investigators (Corn and Graham 1957, Friend, Zileli, Hamilton and Reuther 1958, Griesemer, Barsley, Dragstedt, Wells and Zeller 1953,and Kamijo, Koelle and Wagner 1955) claim, that direct deamination of C.A. by monoamine oxidase plays only a small part in the inactivation of C.A.

Richter (1940) observed, that a large part of orally ingested epinephrine appeared in the urine as sulfoconjugates. Schayer (1951) on the other hand has demonstrated in the rat, that only small quantities of injected epinephrine was excreted as a conjugate in the urine. Armstrong and co-workers (1957) have shown, that an important metabolite of norepinephrine in man was the 3-methoxy-4-hydroxymandelic acid,(VMA). From these findings Armstrong (1957) suggested three possible ways of metabolism of C.A. 1. Deamination of the amines followed by O-methylation; 2. O-methylation preceding deamination; 3. Both reactions at the same time.

Studies on urinary excretion in rats, using labeled metanephrine injected i.p. and recovered within 24 hours, demonstrated the presence of metanephrine in free and conjugated forms and VMA. Similar findings were obtained in animals injected with labeled epinephrine.

These findings indicate, that most of the VMA recovered in the urine results from the deamination of metanephrine in the rat.

The deamination was also studied by observation of the effects of inhibition of monoamine oxidase through iproniazid. In rats pretreated with iproniazid almost all of the administered epinephrine or metanephrine was excreted as free or conjugated metanephrine. The usual amount of VMA was markedly reduced.

This seems to indicate that monoamine oxidase is an important factor in the deamination of metanephrine.

Besides VMA, 3,4-dihydroxymandelic acid (DMA) is another product of deamination of C.A. It is regularily present in the urine of man and the rat. But the amounts of this deamination product are much smaller than those of VMA. They were reported to be markedly elevated in patients with pheochromocytoma (Resnick, Goodall, Kirshner 1958 a & b).

The metabolic fate of Dopamine should briefly be mentioned. Axelrod, Senok and Witkop (1958) report that after administration of Dopamine to rats, a small amount of 3-methoxytyramine (free and conjugated) was recovered in the urine which would indicate O-methylation. Furthermore, considerable

amounts of homoranillic acid were found, as a product of deamination of 3-methoxytyramine. Pretreatment of the rats with iproniazid resulted in production of large amounts of the O-methylation product: 3-methoxytyramine. This seems to demonstrate, that also here monoamine oxidase is involved.

2. The metabolism in patients with chromocytoma.

The investigation of the metabolism of C.A. has been markedly advanced by the discovery of the association of their levels in chromocytomas.

This tumor of the chromaffin tissue of the adrenals or of the extramedullary pheochrome system, is characterized by high release of C.A. The increased hormone liberation into the blood by these tumors and the high content of C.A. and enzymes in the tumors, gave an excellent opportunity for their study. By constant metabolic process of C.A., various metabolites are excreted in the urine in high amounts.

According to Sjoerdsma (1958) the biosynthesis of C.A. proceeds as follows: phenylalanine → tyrosine → 3,4-dihydroxy-phenylalanine (Dopa) → 3,4-dihydroxyphenylethylamine (Dopamine) → norepinephrine → epinephrine. The cellular catalyst in this process is dopadecarboxylase; probably other still unknown enzymes are also present.

The normal levels of epinephrine and norepinephrine in the blood are normally less than 6mcrg./L of plasma, whereas patients with pheochromocytoma show values ranging between 10 and 100mcrg./L of plasma. The urine excretion of norepinephrine and epinephrine is usually less than 100µg/ day (mcrg. day), in normal persons, while it reaches 300-3000 mcrg./day in pheo-

chromocytoma cases. The tumor content of C.A. varies between 500-10,000mcrg./g. Human urine contains physiologically also Dopamine, approximately 100-200mcrg./day. v.Euler (1951) found that also the Dopamine excreted in the urine was increased in patients with pheochromocytoma. Similar findings have been reported in 1956 by Weil-Malherbe and McMillan, specifically in a case of malignant pheochromocytoma. Dopa was also found in some of the pheochromocytomas. The occurence of Dopa and Dopamine in the tumor confirm the concept, that they represent intermediates in the formation of norepinephrine and epinephrine. Armstrong, McMillan and Show (1957) and Axelrod (1957) carried out their studies on metabolism of C.A. in normal and in patients with pheochromocytomas. The latter group of cases provided an opportunity for comparison; large amounts of hormones and metabolites in the tumors themselves, in the blood, the tissues and the urine were found. Some of the tumors demonstrated differences in the content of the hormones. In certain cases pheochromocytomas secreted only dopamine and were called "nonfunctioning" because of the weak pressor ability of this hormone. Some possible differences were also found in the enzymatic process. As far as the metabolism is concerned, the same steps can be observed in pheochromocytomas as in normal persons: O-methylation, deamination and conjugation.

The O-methyl metabolites in pheochromocytomas were found

to be: normetanephrine, metanephrine, VMA, 3-methoxy analogues of Dopamine and two other phenolic unidentified amines. The normetanephrine content of one of the tumors was found to be 25mcrg./g. It is still not known, to what extent the tumors secrete their methoxyamines directly into the blood. Investigations in this field are difficult because of the relatively physiological inactivity of these compounds.

Studies on metabolism in vivo, (Armstrong 1957) showed values of 1,5-3,0 mg./day of VMA in the normal person, while the amount of VMA reached 90, 32 and 12 mg./day in pheochromocytoma patients. la Brosse (1958) found in his experiments high levels of norepinephrine as well as VMA.

The urinary normetanephrine was found to be excreted in a conjugated form with glucuronic acid. Metanephrine was shown to be present in the urine of patients whose pheochromocytomas were producing epinephrine and norepinephrine. Methoxy metabolites as well as deamination products were excreted in high amounts in patients with pheochromocytoma.

Conclusion to Catechol Amines in Pheochromocytoma.

Concluding, it can be said, that the metabolism of C.A. in pheochromocytoma is essentially the same as in subjects with normal hormone levels. Some differences exist in the content of hormones, as there are tumors producing only epinephrine and norepinephrine or only Dopamine. The metabolic products are accordingly presenting the derivatives of the hormone present. The amounts of the C.A. in the tumor, the blood, the tissues and the urine are manyfold higher than those in normal subjects. Catechol-O-methyl transferase is the enzyme found to be most important in the metabolism of C.A. in pheochromocytoma, in normal persons and the rat.

Enzymes

Studies on the metabolism of E and NE in vitro have demonstrated that the enzymes catechol O-methyl transferase as well as amino-oxidase participate in the process. The O-methylation occurred only, when the tissue contained the activated form of methionine-S-adenosyl methionine. (Langemann, Burger, Kagi 1956 and Sjoerdsma, Leeper, Terry and Undenfriend 1959).

There is evidence concerning the presence of an enzyme, which had the ability to transfer the methyl group of S-adenosyl-methionine to nitrogen and also the methyl group can be transferred toward oxygen. (11, 12A). This property has been ascribed to the enzyme O-methyl transferase, which transfers the methyl group A.M (adenosylmethionine) to the 3-hydroxy group of epinephrine. The enzyme was concentrated from the rat liver. It was demonstrated that in absence of AM or Mg++, little O-methylation took place. A number of other cations like Co, Mu, Cd, Te and Ni could be substituted for Mg.

The O-methylation of norepinephrine, dopamine, dopa, DMA and many other synthetic catechols was demonstrated with the presence of the enzyme O-methyl transferase. There was no stereospecificity required. But the enzyme was found to be specific for C.A. SH-binding agents prevented the reaction, suggesting that the reaction takes place by a specific attachment of the enzymes to

the substrates. The metals in the cause are presumed to be acting as intermediates. The enzyme has been found to be present in the liver, lung, kidneys, spleen, small intestine, brain, heart, salivary gland, pituitary, pancreas, aorta, and inferior vena cava. All these organs are capable of O-methylation of the C.A. This finding suggests, that O-methyl transferase is probably acting locally in the transformation of E and NE, since they are exerting their action on all these organs. It is therefore presumed, that O-methyl transferase is the principal enzyme in the metabolism of C.A.

In the nervous system, O-methyl transferase plays an important role in the metabolism of the C.A. Since Norepinephrine has been shown to play an important part in the sympathetic nervous system, it is presumed, that norepinephrine is involved in the activity of the C.N.S. The presence of normetanephrine in the rats brain lead to the conclusion, that the norepinephrine is O-methylated in the brain and further metabolized by deamination. There is also evidence of some other enzymes being involved in this process especially in the formation of S-adenosyl-methionine from methionine. Catechol-O-methyl transferase has been found in the sympathetic and parasympathetic nervous system.

The metabolic pathway for norepinephrine in the central and peripheral nervous systems is presumed to be as follows:
NE - normetanephrine - VMA.

It seems likely that O-methylation is an important factor in the metabolic pathway of C.A. in the nervous system.

In the deamination of metanephrine and normetanephrine to VMA, the presence of monoamine oxidase is evident. Another enzyme was located in the microsomes of the rabbit liver and is presumed to catalise O-demethylation of metanephrine to epinephrine. The process seems to require the presence of oxygen.

CHAPTER V

FUNCTIONS OF CATECHOLAMINES

1. General considerations

Apart from transmission of adrenergic nerve impulses the actions of catecholamines was considered in the past according to the systems affected. Therefore the functions were classified as concerning the cardiovascular system, the respiratory system, the gastrointestinal tract and the central nervous system. However, most of the older classifications do not take into account the recent findings. Koelle (1959) has suggested recently a more accurate grouping of the functions of catecholamines based on distinction between excitatory and inhibitory actions on autonomic effector cells and considered separately the metabolic actions and those on the central nervous system. In the following discussions of the functions of catecholamines we have attempted first to follow the old division of actions on various systems and then to discuss the functions of catecholamines according to the classification of Koelle.

Generally, Noradrenaline produces vasoconstriction, while adrenaline causes vasodilation. However, a vasoconstriction effect has been also observed to follow adrenaline administration, (Ginsberg and Cobbold 1960). It appears that this effect depends on the route of administration of adrenaline. While adrenaline increases the heartrate, the cardiac output and

the coronary flow, Noradrenaline causes bradycardia and has no effect on the cardiac output. Following adrenaline administration the systolic blood pressure rises and the diastolic blood pressure falls. Noradrenaline causes the rise of both, the systolic and diastolic pressure.

Catecholamines have a stimulating effect on the respiration and increase the depth of breathing. The site of the action of catecholamines on the respiratory system is not known. A direct action on the respiratory center has been excluded by the experimental work of Coles (1956). The oxygen consumption increases simultaneously with the increase of respiration.

A linkage of the catecholamines with a receptor site at the cell surface was presumed. Lewis (1954) has shown, that these compounds are active only in the charged or cationic forms and are expected to penetrate the cell surface only very slowly. The excitatory actions of catecholamines like increased heartrate, vasopressor activity, ACTH release, increasing activity of carotid and aortic baroreceptors, E.E.G. activations, ovulation, as well as certain subjective stimulating effects of catecholamines are believed to be activated by this pathway, while the inhibitory actions of catecholamines like bradycardia, inhibition or slowing of reflexes, blocking effects upon peripheral autonomic synapses, blocking of the antidiuretic hormone, relaxation of intestinal mortylity of aorta and uterine contraction are presumed to be brought upon by a reaction of catecholamines

with the cell surface only. On the other hand a reclassification of all functions of catecholamines under the new aspect would be necessary, since the inhibitory actions of catecholamines on the smooth muscle are considered to be associated with accumulation of lactic acid, which action would have to be classified under the third group of functions of catecholamines. The receptors of catecholamines play an important role in this process and it represents a vast field for further investigations. Nicherson concluded from his recent work (1961) that alpha and beta receptors still represent the main groups of adrenergic receptors except those in the heart muscle which act differently. Furchgott (1959) proposed that in addition to the two main receptors classified by Alquist (1948) two more receptors should be added: gamma receptor for the glycogenolytic action and delta receptor acting specifically in the inhibiting function of the intestinal smooth muscle. Furchgott (1959) considers the possibility of a common primary metabolic action of catecholamines leading to the diverse final effects of these agents. Nicherson (1961) however, found some discrepancies in the former concepts concerning receptor-agent combination and reaction. Nicherson (1961) suggests, that there is no specificity of receptors for each separate compound, but rather for a whole group of similar compounds. He also thinks, that the quantity of response does not depend on the number of receptors occupied, that there are as well reversible as irreversible reactions of receptors with the agent and that there are more receptors present in the tissues, than necessary for the reaction to occur. Nicherson has concluded,

that the specific activity of receptors and agents may depend on a gene specificity. The presence of enzymes is required for the transformation of catecholamines in the tissue into all its metabolic stages, thus performing the specific reactions at the same time.

2. The excitatory effects of catecholamines

The administration of epinephrine is generally associated with excitement or nervousness. Patients who for some reason received 0.5-1.5 mgm. of epinephrine intramuscularly or subcutaneously almost invariably complained of some somatic symptoms like muscular tremor of upper and lower extremities, trunk and lips. Increased heart rate, hypernea, salivation, tearing, urinary urgency, cold or tingling extremities, substernal oppression and headache, have also been observed. (Bosowitz & Korchin 1956, Canteril & Hunt 1932, Duremau & Scholander 1956). Purely subjective phenomena following epinephrine administration include excitement, tenseness, exhilaration, restlessness or anxiety and agitation. Hallucinations have been reported by Hofer 1957. While attempting to distinguish between "true" and "cold" emotions, it was found by Canteril & Hunt (1932) that most subjects reacted with cold emotions, that is, exciting, pleasant and unpleasant feelings equally distributed, while true emotions of fear and anguish were not frequently noted. Usually, the reactions to epinephrine were found in persons with hyperthyroidism or in emotionally labile subjects. On the other hand even high dosages of epinephrine and marked hypertension would not cause any similar

reactions in "normal" subjects. (Goldenberg & Aranow 1950). It appears that epinephrine most probably has a certain arousing or exciting effect depending on the personal excitability of the subject.

Norepinephrine was observed to lack those central excitatory effects. (Rothballer 1959). However, it must be taken into consideration, that norepinephrine can be administered only in considerably lower dosages and would probably have similar effects in higher dosages.

Epinephrine showed definite E.E.G. activation and behavioral arousal in the anesthetized and unanesthetized animal, using small dosages of 2-5 mcrg./Kg. The effect was apparent within hours after the injection.

The stimulating effect of epinephrine on release of cortico-adrenal hormones was first observed by Vogt (1944). In her experiments Vogt demonstrated, that the life of adrenalectomized rats has been considerably prolonged, if infused with blood from the adrenal vein of dogs receiving epinephrine intravenously. A fall in adrenal ascorbic acid following 200 mcrg. of epinephrine (intravenously or subcutaneously) was demonstrated by Long and Frey (1945). The effect was absent in hypophysectomized animals. From these findings the authors concluded, that the pituitary ACTH was the acting hormone in the cause. Farrell and McCann observed a rapdi ACTH release into the blood stream

following an injection of epinephrine. The blood from these rats produced the fall in adrenal ascorbic acid in hypophysectomized animals. Epinephrine alone seemed to be ineffective. Norepinephrine showed a markedly weaker eosinopenic effect. Recant (1950) has demonstrated, that ACTH, cortisone and epinephrine were producing eosinopenia in man and the animal. Cortisone alone produced this effect without pituitary or adrenals, but ACTH required the animals.adrenals.

Epinephrine produced eosinopenia in the presence of adrenals, pituitary and anteriour hypothalamus. The authors concluded, that epinephrine was stimulating ACTH production in the pituitary and that the pathway of this mechanism leads through the hypothalamus. Muehrcke (1952) has demonstrated for the first time, that the activation of the pituitary-adrenal axis was not necessary for eosinopenia. He attained this effect with 0,3 mg. of epinephrine subcutaneously in patients with bilateral adrenalectomy and orchidectomy, maintained on cortisone. Henry (1953) et.al. concluded, that epinephrine was capable of producing eosinopenia, but that 11-oxy steroids were necessary to be present. With the methods of measurement of corticoids in blood and urine many authors postulated, that epinephrine did not raise the level of these hormones, nor that of ACTH and that epinephrine eosinopenia was

not due to this factor. The direct stimulating effect of epinephrine is impaired in conditions like cold, insulin, laparotomy or histamine injection. Vogt (1952) demonstrated, that the adrenal medulla was not necessary for the ACTH response to physical stress like cold, histamine, hemorrhage, etc. Guillemin (1955) concluded, that neither epinephrine or norepinephrine were indispensable for the ACTH release in stress. Conclusively it can be stated, that epinephrine causes the release of ACTH in most species. In the rat this was postulated by the demonstration of the fall of adrenal ascorbic acid and cholesterol. The adrenal medulla is not essential in the release of ACTH in stress. Epinephrine-induced eosinopenia represents a direct effect of the hormone, requiring the presence of cortical steroids. The action of epinephrine on the reticular activating system can not be denied.

Epinephrine and norepinephrine injected into the anterior pituitary gland or third ventricle is stimulating ovulation in rabbits. Intravenous or intracarotid administration is ineffective. Whether the hypothalamus or the pituitary is the primary site of action is still disputed. Dibenamine, Dibenzyline, atropine, Nembutal, Banthine, morphine and alcohol exercise a blocking effect on this action. Priscoline and Regitine have failed to produce the blocking effect. It is not known yet,

whether these drugs can pass the blood - brain barrier. It has been postulated, that this stimulating effect of C.A. on ovulation is mediated through adrenergic mechanisms within the hypothalamus, where those hormones are present in great quantities. It must be added, that most of the drugs have ambiguous actions and the effects found have to be treated with caution.

Epinephrine and norepinephrine stimulate many of the effects of electrical stimulation of the brain stem, reticular formation, including EEG activation, spinal motor fascilitation and reflexes involving the brain stem. There is evidence to suggest, that epinephrine acts directly on the brain stem, the mesencephalon and the posterior hypothalamus. These effects are blocked by administration of anesthesia.

3. The inhibitory actions of Catecholamines

The following actions of catecholamines have been classified as inhibitory; bradycardia, bronchial dilatation, reflex inhibitory action, blocking effect upon peripheral autonomic synapses, blocking of ACTH release, antithyroid and antidiuretic hormone activity, relaxation of the intestinal aortic and uterine smooth musculature. As it will be pointed out later under discussion of Bülbring's theory, some of these functions can be considered as part of a dual action of adrenaline. Therefore, the following comments on inhibitory actions of catecholamines are restricted to the effects on the intestinal smooth muscle in view of the authors interest in the role of catecholamines in gastric secretory mechanism.

Mohme-Lundholm (1953) has shown in experiments on rabbit's intestine that adrenaline is capable of producing a decrease of tone and a total inhibition of the contractions. This effect was evident after administration of small dosages of adrenaline (20 gamma in 20 ml. of a solution of adrenaline 1:750,000 - 1:2,800). At the same time a marked increase in lactic acid content of the gut was observed. This increase persisted throughout the relaxing action of adrenaline. In different experiments lactic acid was added to the preparations. This has caused a complete inhibition of tone and peristaltic activity of the gut. When tone and peristalsis were only partially blocked by adrenaline, the content of lactic acid was decreased. In experiments in which lactic acid was added

to small amounts of adrenaline, the inhibitory effect reached the same level as with higher dosages of adrenaline. Addition of sodium bicarbonate in order to inhibit lactic acid action, necessitated an increase of the dose of adrenaline and lactic acid to achieve the same effect. Ergotamine and Ephedrine, $CuCl_2$ and NaF inhibited the relaxing action and the lactic acid stimulating effect of adrenaline. Lactic acid formation was found in preparations devoid of the muscular substrate. Whether the relaxing effect of adrenaline on the intestinal smooth muscle is a consequence of lactic acid accumulation or whether the relaxing action and the lactic acid stimulation are two separate functions of adrenaline occuring simultaneously, is not well known yet.

. Bülbring's theory on dual functions of Adrenaline in Experiments on guinea pig's intestinal smooth muscle.

Bozler (1940) and several other authors have observed, that in the visceral smooth muscle the effect of adrenaline and sympathetic stimulation was variable, diphasic, excitatory or inhibitory and that these functions could be reversed according to the condition of the tissue. This observation has been recently evaluated by Bülbring (1959) who postulated, that the dual action of adrenaline would depend on the condition of the electric potential of the cell membrane: adrenaline produces a contraction of the smooth muscle, when there is a depolarisation; accordingly a relaxation takes place, when hyperpolarisation is present. The exact study of this problem has only been made possible after the development of the sucrose

gap method suggested by Stämpfli (1954). This method allows an accurate measurement of electric potentials and ion movements on the cell membrane. This way Bülbring (1959) was able to demonstrate the mechanism of the dual action of adrenaline on the guinea pig intestinal smooth muscle. Bülbring (1959) postulates, that one of the actions of Adrenaline is a direct one, on the cell membrane, causing an increased permeability for ions, sensitization and depolarisation of the cell membrane. The second action is indirect and presumably has a metabolic character, mediated through an increase of phosphorylase activity, thus accumulating energy and stabilizing (relaxing) the cell membrane. A stage of hyperpolarisation on the cell membrane is achieved in this phase. With this accumulated energy the cell has a dual potence: it can use the energy for a contraction, or it can use it for active ion transportation into the cell, which process also requires energy.

This hypothesis of Bülbring's concerning the action of adrenaline on the smooth muscle is to be considered as a result of two opposing actions: in those types of smooth muscle in which adrenaline produces a relaxation, the metabolic, indirect, effect of adrenaline predominates, the action potentials are abolished. With the direct action of adrenaline the membrane permeability predominates and determines the contracting effect on the cell. This hypothesis postulates also, that adrenaline is capable of exciting both actions in each type of smooth muscle. The nicitating membrane and the pregnant uterus of the cat is quoted as an example of a direct, excitatory

effect of adrenaline on these smooth muscles. Bülbring calls attention to the fact, that the relaxing effect of adrenaline on the intestinal smooth muscle should not be considered as an inhibitory effect on the contractile mechanism, but as a result of cessation of electrical activity, due to an increased rate of ion transportation. The latter is to be considered as a consequence of an increased rate of energy supply. Bülbring (1959) concludes, that the membrane of the intestinal smooth muscle is a very unstable structure. It depolarizes continuously and discharges permanently electrical impulses. It seems, therefore, that it never reaches its full resting potential. Adrenaline will under these circumstances stimulate those processes, in which the membrane stabilizes to reach its resting (relaxing) potential values. Adrenaline causes the following electrical changes on the intestinal membrane: it stops spontaneous activity, it renders the preparation electrically inexcitable; it causes hyperpolarisation. Thus relaxation is the expected consequence. At the same time the phosphorylase activity is increased by adrenaline. This results in accelerated glycogen breakdown and activation of ion transportation. Especially affected is the "sodium pump".

Ellis (1959) demonstrated, that smooth muscles which were excited by catecholamines showed a lack of potassium, while the smooth muscle elements inhibited by catecholamines presented an accumulation of potassium. Born and Bülbring (1956) demonstrated, that adrenaline increases the potassium uptake of the intestinal smooth muscle. This is also the case after

exposure to acetyl choline and histamin. The sodium exchange in the intestinal smooth muscle was found to be fifty times faster than that of potassium. This probably is of some importance for the maintenance of the membrane's electrical potential. Sodium uptake is considerably decreased and the sodium loss is almost double in the presence of adrenaline. It can be assumed that adrenaline produces the relaxing effect by stimulating one reaction in a chain of metabolic cycles, finally energy is supplied for an "electrogenic sodium pump", stabilizing the membrane.

4. Actions of Catechol Amines on Metabolism

Considerable amounts of literature exist concerning the metabolic effects of catechol amines and related sympathomimetic amines.

The first metabolic effect of catechol amines to be described, was that on carbohydrate metabolism, (Blum 1901). Later reports have dealt with the effects on protein metabolism (Reid 1941), amino acids (Rose 1935), lipids and other organic nitrogenous substances (Page, Pasternak and Burt 1931), the effect on the calorigenic action (Boothby and Sandiford 1923) and on the oxygen consumption rate, (Belawenez 1903, Juschtschenko 1909). The above listed metabolic actions will be considered in more detail.

A. Carbohydrate Metabolism

Blum (1901) was the first to describe the so called adrenal diabetes. Since that time epinephrine hyperglycemia has been studied in detail by several authors. (Cori 1931, Cori 1953, Trendelenburg 1924 and 1934).

One of the best known metabolic effects of catechol amines is the hepatic glycogenolysis by epinephrine, which is responsible for an increased level of glucose in the serum. As the liver glycogen released is insufficient to cover the high output of carbohydrates caused by epinephrine, gluconeogenesis is stimulated at the same

time, taking place by conversion of fat or proteins or other organic substrates. Simultaneously with the liver process and evidently caused by the same mechanism, increased muscle glycogenolysis takes place, causing accumulation of large amounts of lactic acid in the muscle. The lactic acid from the muscle has been shown to be responsible to a great part for hepatic gluconeogenesis through the lactic acid cycle. (Cori 1931, Cori 1945-46).

In this connection glucagon has been found to have similar actions as epinephrine, with the difference, that glucagon does not affect the glycogenolysis of the muscle but probably acts only directly on the liver levels. The recovery of liver glycogen after glucagon administration is more prolonged, than following epinephrine injection. The hyperglycemia itself is of shorter duration in glucagon. This indicates that epinephrine has an adaptating action on the blood carbohydrates as shown in partially pancreatectomized rats by Ingle, Beary and Purmalis 1953.

It has been presumed that part of the high glucose levels in epinephrine administration could be derived from fat transformation (Soskin 1941, Stadie 1945). However, it has been shown at the same time, that glucose was not formed as an end product of fat glycogenolysis (Mirski 1942).

Wiechmann (1927) suggested that decreased peripheral

glucose utilisation is one of the factors responsible for hyperglycemia in epinephrine.

He presumed that epinephrine depressed the glucose assimilation in the peripheral tissue and proved it, by the differences found in comparisons of arterious and venous levels of glucose. These differences have been greater after glucose administration, than following epinephrine injections. Miechmann's observations have been confirmed in several experimental studies on man and in animals, (Amatuzio & coworkers 1954, Cori & Cori 1929, Cori, Fisher & Cori 1935, Somogyi 1950 & 1951, van Itallie & Bentley 1955).

According to these studies, the reduction of peripheral utilisation of glucose seems to be appliable only to cases of high glucose levels produced by epinephrine or during alimentary glucose depletion, but not to resting serum glucose levels. The fact that epinephrine produced a potentiated hyperglycemia in combination with glucose or glucagon, was also explained by the above authors as a reduction of peripheral assimilation of glucose.

Tolerance tests by Amatuzio (1954) have demonstrated that epinephrine greatly reduced the rate of blood glucose removal. Ingle and Mazamis (1949) could demonstrate that in eviscerated rats, infused with glucose

and insulin, epinephrine and isoproterenol administration reduced glucose assimilation. In the isolated muscle and in the isolated rat diaphragm, the depression of glucose assimilation by epinephrine administration was shown to be as high as 30% (Walaas 1952 & 1955).

In other experiments with large variations of insulin concentration the reduction of glucose assimilation by epinephrine was 40% (Candela & Candela 1955). Whether epinephrine hinders glucose uptake by the liver has also been questioned. Generally there is an increase in tissue utilisation of glucose, when the blood sugar level is increased. Lundsgaard & coworkers(1939) demonstrated clearly in their work that at low blood sugar levels epinephrine had little effect on utilisation of glucose, rising with increasing glucose levels. This applies also to low epinephrine levels, which have no effect on blood sugar; while higher dosages of epinephrine which cause a rise in blood sugar, also produce a decrease of assimilation of glucose. Ellis (1959) considers, that under certain circumstances the calorigenic effect of epinephrine may be sufficient to increase the over all utilisation of glucose. In this connection the experiments of Himsworth and Scott (1938), may be considered according to which the rate of fall of glucose was increased by epinephrine in rabbits, with the liver excluded from circulation.

Ingles and Nazamis (1949) could demonstrate, that glucose utilisation was increased by epinephrine in eviscerated rats. It has been shown that epinephrine increases glucose consumption during exercise (Dill & Edwards 1935). Ellis (1959) postulates, that, since insulin increases glucose utilisation in exercise, the decreasing effect of epinephrime on glucose utilisation can be overcome by exercise. In this case epinephrine hyperglycemia and muscular exercise are acting synergistically in increasing carbohydrate metabolism.

Differences in species in response to epinephrine hyperglycemia have been found. (Somogyi 1950, Greuel & Sachsse 1955, Cohen & Portman 1951). These concern the receptor affinity for epinephrine in the liver. Rabbit liver is about 8 times more sensitive than rat liver. In crocodiles intracardiac application of epinephrine produces a hyperglycemia which persisted for several days (Ellis & Anderson 1952).

Intraspinal, intrathecal and intraperitoneal administration of epinephrine was found to be equally effective as intravenous application.(Kubicek & Gottke 1953, Leimdorfer 1950, Ellis & Anderson unpublished observations).

Hyperglycemia was found to take place in animals with denervated hearts, as well as in animals with ab-

olished hepatic arterial supply. Seasonal,dietary, and sex differences in epinephrine hyperglycemia in man have been observed (Altschule & Siegel 1951). Women show higher epinephrine hyperglycemia than men (Enochsson & Gjertz 1934). Patients with certain mental diseases or epilepsy show poor response to epinephrine. The same reaction was seen in patients with chronic psychoses. Frontal lobotomy restored the epinephrine action completely, independently of the improvement of the mental disease. Mongoloid individuals exhibited a relative insensitivity to epinephrine. This has been attributed to a defficient pituitary activity. Patients with hepatic disease or diabetes showed reduced hyperglycemic reaction to epinephrine.(van Itallie & Bentley 1955). van Itallie and Bentley (1955) proposed a liver function test by administration of subcutaneous epinephrine and glucagon. In individuals with liver disease the rise in blood sugar was somewhat higher than in normal persons. This seems to demonstrate the ability of the liver to convert lactate to glucose, which is decreased in liver patients.

A. Effects on Carbohydrate Metabolism in Various Tissues.

Liver. A difference of opinion existed whether epinephrine decreased or increased liver glycogen until it was shown by Evans, Tsay and Young 1931, that liver glycogen was little above or at normal levels one hour after subcutaneous injection of epinephrine. However, only with high dosages of intravenous epinephrine a continuous depletion of liver glycogen could be demonstrated. (Samson 1933). Recent experiments on human subjects have provided the evidence, that epinephrine increased hepatic glucose output. (Bearn, Billing & Sherlock 1951). To the contrary of former opinions, that intraportal infusion of epinephrine does not have a direct glycogenolytic effect on the liver, it has been shown in recent experiments, that epinephrine injected into the portal system in higher dosages causes hepatic glycogenolysis.

Perfused livers of frogs, cats, dogs and liver slices of rats, rabbits, cats and dogs showed increased glucose output in response to epinephrine. (Morita 1914-1915, Lesser 1920, Lundsgaard 1939, Sutherland & Cori 1951, Ambrus 1955).

Kepinov (1949) claimed, that epinephrine would be active only when anterior pituitary hormone was present. Epinephrine hyperglycemia was lower in

hypophysectomized animals (Lane & Bodo 1952, Lopes & co-workers 1954) but the reduced hyperglycemic effect of epinephrine in these experiments could be attributed to a deficiency of adrenocortical hormones following hypophysectomy. (Harvey, Wang & Nickerson 1952). Underhill and Closson (1906) postulated, that epinephrine would also diminish glucose uptake by the liver. This has been confirmed by Crane and Sols (1953) & Weil-Malherbe and Bone (1951). These investigators believe, that there are several separate enzyme systems involved in the transformation of glucose to glucose-6-phosphate and in the liberation of glucose from glucose-6-phosphate. The latter probably controls the rate of glucose liberation from hepatic cells and it may inhibit glucose uptake by inhibiting hexokinase. Quantitative studies on specific activity of glucose-6-phosphate have not been carried out. But the latter, being a source of secreted glucose, may approach the specific activity of the glucose supplied to the liver, when glucose uptake is increased as in Insulin, or it may modify the specific activity of the liver glycogen, when glycogenolysis is increased as following epinephrine. (Renold & Hastings 1953). Teng and co-workers (1952) claimed, that following glycogen deposition in the liver from glucose, epinephrine glycogenolysis was less effective. Experiments with glycogen deposition

from other sources such as fats and proteins, have also been carried out. (Stetten & Kline 1945). In such cases epinephrine may act only as glycogen depleting substance raising blood lactate and setting the stage for more accellerated glycogen deposition. However, glycogen formed from injected lactate was not reduced by epinephrine. (Carrasco-Formiguera 1950).

Muscle. Fisher & Krebs (1955) demonstrated for the first time the conversion of inactive into active phosphorylase in cell-free preparations, by adding muscle phosphorylase. Although the effects of epinephrine on muscle glycogenolysis and on lactic acid formation have been demonstrated repeatedly, there seems to be a difference of opinion concerning the effect of epinephrine on glucose assimilation by the muscle. (Cori & Cori 1929, Hagemauer & Cori 1934, Nachmansohn & Nachmansohn & co-workers 1936-1937, Tolstoi & Loebel 1923- 1924). Walaas & Walaas 1950 showed in their experiments in vitro with rat's diaphragm, that glycogenolysis and lactic acid formation was increased and the glucose uptake decreased with epinephrine. These findings have been confirmed by Ellis & Anderson 1954, Sutherland 1952 and Walaas 1955. Fructose and mannose uptake are also inhibited by epinephrine in the rat's diaphragm, since those sugars are

also assimilated by hexokinase. (Walaas 1955).

Muscle extracts from epinephrine-treated rats used less glucose than extracts from control animals. There was no difference in the hexosemonophosphate in normal and epinephrine treated animals. Since sodium fluoride diminished glucose utilization in control animals, it was concluded, that ATP is the controlling mechanism in muscle glycogen metabolism and that ATP was amenable to changes by epinephrine. (Cohen & Needham 1950). Crane and Sols 1953 suggested, that the increased glucose-6-phosphate concentration in the muscle treated with epinephrine, was the reason for reduced glucose assimilation. Exercise is known to increase glucose uptake from the muscle.(Flock & Bollmann 1940).

This may be explained by permeability changes but probably also by changes in phosphorylase activity or glucose-6-phosphate concentrations. Epinephrine does not change oxygen consumption in the rat's diaphragm. (Walaas & Walaas 1950). Stadie and co-workers (1951) exposed rat diaphragms to epinephrine at 25° C and proved that epinephrine-treated diaphragms synthesized less glycogen than control diaphragms. This was elucidated by further experiments and it was found, (Ellis & Anderson 1955) that 25° C was the optimal temperature for the action of epinephrine on rat's diaphragm. It has been shown, that free intracellular

muscle glucose is increased during fast glycogenolysis caused by epinephrine. (Cori, Closs & Cori 1933). After epinephrine injection muscle glycogen decreases progressively while blood sugar is elevated. In the rat's diaphragm glycogenolysis occurred during the first five minutes. No further decrease in glycogen occurred until the end of one hour. Still there was some difference of glycogen content in control diaphragms, as a consequence of continuous glycogen concentration which was partially inhibited by epinephrine. (Walaas 1955).

Heart. Increased glycogenolysis in the heart muscle by epinephrine was demonstrated in vivo and in vitro. (Patterson & Starling 1913-14, Chang 1937). However the effect of epinephrine on heart glycogen appears to depend greatly on dosage and condition of the heart at the time of hormone administration. It was found, that 0.5 mgm. of epinephrine per kg. of body weight was most effective and that amaerobic or cyanide treated hearts gave best results. (Bogue & Evans 1939, Cohen & Clarke 1951).

The glycogenolytic effect of epinephrine on the heart muscle was evidently only transient, to the contrary to other muscles and organs. (Dahyun & Luck 1929-30). Decreased glucose uptake was demonstrated

in isolated epinephrine-treated hearts. (Loewe and co-workers 1914). There was evidence of increased glucose uptake in epinephrine-treated hearts. (Patterson & Starling 1913-14, Wilenko 1913). These results can only be affirmed by comparison with glucose uptake by the muscle during exercise. The depressor effect in such cases may be counteracted by the effect of work on muscle glucose assimilation. In the heart too, phosphorylase is involved in the epinephrine acting mechanism. In heart slices treated with epinephrine, glycogen was reduced, phosphorylase activity increased and the concentration of glucose-6-phosphate also was increased. (Ellis & Anderson).

B. Cellular Mechanism of Action of Epinephrine on Carbohydrates

Cori (1940) suggested that the mechanism of action of epinephrine on liver glycogen output was caused by an increase of concentration of active enzyme (phosphorylase) in the cell. However, all his experiments on the subject gave negative results. In 1951 Sutherland and Cori have demonstrated, that epinephrine increased liver phosphorylase in vitro, in slices and also in vivo. The two investigators established, that in the chain-reaction (glycogen $\longrightarrow$ glucose-1-phosphate $\longrightarrow$ glucose-6-phosphate-glucose)the first reaction to take place was rate limiting and that epinephrine increased the concentration of glucose-1-phosphate and glucose-6-phosphate. They also established, that phosphorylase was the site of action for epinephrine in activating glycogenolysis.

Phosphorylase activity was increased by epinephrine. It has been also found, that phosphorylase activity in liver and muscle was present in a dynamic state: active phosphorylase $\rightleftharpoons$ inactive phosphorylase an inactivating enzyme and by an epinephrine catalyzed system for reactivation of phosphorylase. Sutherland 1951 could show, that epinephrine was ineffective, when the liver cells were damaged by freezing, homogenization, anoxia, cyanides, alcohol and fenorides.

From these experiments Sutherland and Cori concluded, that the cell must be the site of action of epinephrine utilizing the above mentioned mechanism.

It has also been postulated, 1) that epinephrine has to be combined with the cell by certain receptors in order to be active, 2) that there was energy required for the reactivation of phosphorylase (Gaddum 1950), 3) that Adenosinetriphosphate was important for phosphorylase activation.

Sutherland and co-workers 1956showed, that phosphorylase activation was stimulated by epinephrine and glucagon, with magnesia and ATP added to liver homogenates.

C. Interaction of epinephrine and other hormones.
Epinephrine and Insulin in carbohydrate metabolism.

Insulin antagonizes the initial fall in liver glycogen as well as the rise in blood sugar of rats treated with epinephrine. (Cori & Cori 1930). Some authors Lundsgaard & co-workers 1939, and Ambrus & co-workers 1955, could not demonstrate any such effect of insulin on epinephrine in their experiments carried out on rabbit or dog livers. The findings can not be explained at the present. Cori(1927-28) suggested that epinephrine counteracts insulin hyperglycemia by antagonizing the effect of insulin on glucose utilisation. The reduced peripheral utilisation fortifies the epinephrine induced elevation of hepatic glucose production. Cori and Buchenwold (1930) demonstrated epinephrine -insulin antagonism on frog muscle under aerobic and anaerobic conditions. Concerning the site of action of each hormone, it was reported (Riesser 1947) that insulin antagonized the glycogenolytic effect of epinephrine on the rat diaphragm, suspended in glucose free media. Experiments on rat diaphragm with different insulin and constant epinephrine concentration, demonstrate, that the antagonism of the two hormones is rather complex. This is not just a summation of the effects of each hormone, nor is the effect dependant

on the concentration of epinephrine or insulin. The percentage of inhibiting effect of epinephrine on glucose uptake seems to be constant, independent of the concentration of insulin.(Walaas 1955). The effect of epinephrine and insulin on adipose tissue glycogen were antagonistic, when administered simultaneously. (Tuerkischer & Wertheimer 1946). Buckaert and Duve (1947) call attention to the fact, that each of these antagonists increase the cellular concentration of hexosemonophosphate which plays an important role in absorption of glucose and the disposition of glycogen.

Epinephrine and Adrenocortical Hormones in Carbohydrate Metabolism.

Adrenocortical hormones seem to be very important for some of the usual responses to epinephrine. According to Eiselt's report (1910) epinephrine glucosuria does not occur in patients with Addison's disease. In adrenocortical insufficiency a diminished hyperglycemic response to epinephrine could be observed. (Bloom & Russell 1955, Collip, Thomson & Toby 1936-37, Safford & co-workers 1946). However there was a greater fall in muscle glycogen associated with normal hyperlacticacidemia. (Bloom & Russell 1955, Collip,Thomson & Toby 1936-37, Winternitz & Long 1952). In adrenalectomized rats, epinephrine increased the metabolism and body temperature. (Ring 1938).

This observation, together with the findings that epinephrine usually elevates blood lactic acid in adrenalectomized animals, supports the assumption, that the calorigenic effect of epinephrine is a result of hyperlacticacidemia. (Lundholm 1949). There is usually a rapid resynthesis of glycogen in the liver after epinephrine administration. This is prevented in adrenalectomized rats. (Winternitz and Long 1952). Cortical hormones administered to normal, adrenalectomized or hypophysectomized rats antagonized epinephrine glycogenolysis in certain muscles. (Winternitz and Long 1952, Wortman and Leonard 1953).

Cortisone increases gluconeogenesis, it also potentiates epinephrine hyperglycemia and increases glucose uptake by the muscle. Desoxycorticosterone potentiates epinephrine action on muscle glycogenolysis in intact or adrenalectomized rats. (Taylor and Leonard 1956). Desoxycorticosterone has little glucocorticoid activity of cortisone and it was found to activate glycogenolysis. (Bozovic and coworkers 1949). Adrenal steroids seem to be more important for the maintenamce of normal glycogen concentration in the liver, while the pituitary hormones seem to be important for the muscle glycogen levels. (Engel 1953, Long 1952). Absence of adrenals dimishes epinephrine hyperglycemia and does not

influence epinephrine hyperlacticacidemia. Increased loss of muscle glycogen following epinephrine in adrenalectomized animals, can be interpreted as a reduced blood glucose which is not compensated by the missing adrenal, in order to counteract glycogenolytic effect of epinephrine on the muscle. The same explanation can be given for the autagonism of glucocorticoids toward epinephrine glycogenolysis in the muscle, on the basis of potentiated epinephrine hyperglycemia in cortison pretreated animals.

D. Miscellaneous actions of catechol amines on metabolism

Potassium Metabolism. Hypercalcemia preduced by epinephrine was found to be diminished in adrenalectomized cats. However, the hyperkalemic response to epinephrine appears to be inversely related to plasma potassium levels.(Verzar 1951). When plasma potassium was elevated by administration of cardiac glycosides, the potassium response to epinephrine was minimal. The administration of desoxycorticosterone to adrenalectomized cats restored hyperglycemic and hyperkalemic response to epinephrine. (Verzar & Somogyi 1951). In adrenalectomized animals epinephrine was found to reduce elevated blood potassium levels.

Concerning the action of epinephrine and pituitary hormones on metabolism,(Dury and co-workers 1950), epinephrine was found to produce lesser hyperglycemic response in hypophysectomized, than in normal animals, especially when the liver glycogen was within normal limits.(Ichijo 1934, Russell and Bennett 1937). On the other hand the pituitary appears not to be the main factor in the mechanism involved, since the administration of cortisone to hypophysectomized rats restored the hyperglycemic effect of epinephrine.(De Bodo and co-workers 1952). Cortical hormones were found to restore the blood sugar levels and liver glycogen in fasted, hypophysectomized rats. The muscle glycogen could not be restored in a similar fashion. (Long 1952).

Leonard (1955) has demonstrated that epinephrine produces the same percentage of muscle glycogen depletion in normal as in hypophysectomized rats. Growth hormone reduces glycogenolytic effect of epinephrine on certain muscles in hypophysectomized rats only. Hypophysectomized rabbits showed the usual increase of blood lactate in response to epinephrine. (Cope & Thompson 1936-37). The glycogenolytic and hyperlacticacidemic effect of epinephrine in hypophysectomized animals appears to be greatly depending on whether the animal is nourished or fasting. Fasting of four hours duration will deplete the muscle of glycogen. (Russell & Cori 1937).

Kepinov (1937) postulated, that the pituitary hormones are required for epinephrine activity in glycogenolysis of hepatic cells. Kepinov could demonstrate, that epinephrine increased glucose production in liver slices, when pituitary extracts were added to the perfusion. Epinephrine produced a lesser increase of oxygen consumption in patients with Simmond's disease, when compared to normal subjects. (Horstmann 1954).

Enockson & Gjertz (1934) observed, that epinephrine produced a more pronounced hyperglycemia in men than in women. Administration of heterologous sex hormone slightly increased epinephrine hyperglycemia (Kluken & Latz 1953).

Testosterone was found to antagonize the glycogenolytic effect of epinephrine on the muscle, (Taylor & Leonard 1956) while progesterone was ineffective. Concerning epinephrine and thyroid hormone on metabolism it can be stated, that the thyroid controls several metabolic effects of epinephrine. Epinephrine hyperglycemia was increased by thyroid hormone administration, reduced by thyroidectomy and restored again by thyroid hormone donation. (Burn & Marks 1925, Comsa 1950, Trendelenburg 1953). Chronic administration of thyroid to rabbits reduced epinephrine hyperglycemia by depleting liver glycogen. (Burn & Marks 1925).

Rabbits showed to be more sensitive than dogs and cats. The effect of epinephrine on muscle glycogenolysis was increased by thyroid and reduced by thyroidectomy. (Collip, Thomson & Toby 1936-37, Leonard 1955). Effects of thyroid hormone on hyperlacticacidemia are not well understood. The calorigenic effect of epinephrine was markedly diminished by thyroidectomy and increased by thyroid administration. (Abderhalden & Gellhorn 1925, De Vissher 1946-49). The calorigenic effect of epinephrine was most pronounced in hyperthyroidism and least in hypothyroidism cases. (Horstmann 1954). Oxygen consumption was also found to be increased with addition of thyroid hormone to epinephrine. (De Vissher 1949).

Comsa (1948 & 1950) found that thyroidectomy reduced and thyroid administration increased the response of epinephrine in respect to: creatin metabolism, reduction of blood cholesterol and hyperglycemia. Brewster (1956) postulated, that thyroxin acts in the way of sensitizating the tissue to epinephrine. Thus, the thyrotoxic effect on the heart rate, cardiac index and arterial pressure, do not represent an effect of thyroxin itself, but are due to an augmented sensitization of tissue to epinephrine and norepinephrine. This can be demonstrated by the fact, that denervated hearts result in considerable protection against thyroxin intoxication. (Krayer & Sato 1928). Dibenzyline, an adrenergic blocking agent, prevented the calorigenic effect of low dosages of thyroxine. (Holtkamp & Heming 1953). It is not yet known, whether Dibenzyline interferes with the calorigenic effect of epinephrine. An increase in blood Histamine was found following epinephrine administration. (Baur & Staub 1949). Leukocytosis was also present as reported by the same investigators. Whether the increase of blood platelates could be considered as a sequence of epinephrine administration is questionable, since the platelates contain most of the blood Histamine.

The tolerance to epinephrine administration is different in various species. Abderhalden & Gellhorn (1925) observed, that some mice would become tolerant

to epinephrine, while others not. Thyroid in any case would raise the tolerance level to epinephrine. Esex (1952) reported that dogs would become resistant to lethal dosages of epinephrine. Repeated epinephrine hyperglycemia leads to reduction of the response. Diet high in carbohydrates reduces the hyperglycemic response to epinephrine.(Altschule & Siegel 1951). Rabbits were found not to develop tolerance to epinephrine, inspite of daily administration.(Bennett 1955).

Influence of Catechol Amines on Fat Metabolism.

B. (a) Fat Catabolism.

The most pronounced difference observed between diabetes mellitus and adrenal diabetes was the fact, that there was no ketonuria in the latter. (Blum 1901, Zuelzer 1909).

Cori and Cori (1928) concluded in their review of the literature that the influence of epinephrine on fat catabolism was of considerable significance. Many investigators observed, that there was no change in urinary ketones in normal nourished subjects, but that there was a decrease in blood ketones when glucose and lactates were increased. (Dill & co-workers 1939, Assmussen and co-workers 1940, Göbell and Kranse 1941, Konrad and Loew 1951). There was an increase in keton bodies after epinephrine injection, when hepatic glycogen was low, or when glycogen utilisation was impaired by a glycogen storage disease. In all experiments carried out on perfused cat's liver, on rat's liver slices and in liver homogenates, an increased ketone formation was observed following epinephrine administration. (Blixenkrone-Möller 1938, Harel-Ceddaha 1955, Haugaard & Haugaard 1954, Niwa 1954).

Epinephrine reduced the amount of radioactive acetate incorporated into the fatty acids of rat's

liver slices. (Haugaard & Stadie 1953). The same effect was obtained with glucagon administration. (Haugaard & Haugaard 1954). From the experiments outlined above it can be postulated, that the increased production of acetoacetate and diminished fatty acid synthesis by the liver, was a result of increased oxidation of fat. This view was further supported by observations of Harel-Cedaha (1953 and 1955), who found that epinephrine cytochrome C and ATP increased the oxidation of octanoate in rat liver homogenates. In intact animals epinephrine elevated ketone production only when hepatic carbohydrate metabolism was inadequate. In vitro epinephrine was found to cause a marked activating effect on ketone production. Accellerated depletion of glycogen from the liver was observed after epinephrine and glucagon administration in vitro, Whether epinephrine has a direct catalytic effect on fat metabolism or whether it is only secondary to the glycogenolytic effect, is still open for investigations (Ellis 1958).

(b) Fat Transport.

Several investigators expressed their opinion that there was an increase, a decrease or no change in the blood fats, following epinephrine administration. (Griffith & co-workers 1949).

Recent investigators show, that there are diverse changes in blood fat after epinephrine, concluding that the changes were depending on the dosage of epinephrine, the duration of action, the level of glycogen in the liver and of fat content in the blood. (Ellis 1958). According to Kaplan & Grant (1955) there was an increase of blood phospholipids of total cholesterol and of fatty acids in the blood after repeated epinephrine administration. In chickens a decrease of blood fats was observed after epinephrine injection. (Mac Vicar & Heller 1941). Under certain conditions, individual lipid fractions were modified by epinephrine, having the total lipid concentration unchanged. (Dury & Treadwell 1953, Dury & Treadwell 1955). Epinephrine increased the concentration of unesterified fatty acids in the blood. (Dole 1956, Gordon & Cherkes 1956). Similar reports were published concerning the changes of cholesterol by epinephrine. (Bruger & Rosenthal 1934).

Concerning fat transportation, epinephrine increased lipids in the liver and reduced the lipides in fat tissues. (Clément & Schaeffer 1947, McKay 1937). Some investigators reported, that chronic administration of epinephrine increased blood phospholipid total cholesterol and fatty acids. (Kaplan & Grant1955).

Others report a decrease in blood fat fractions following epinephrine administration. (Page, Pasternak & Burt 1931, Schaeffer & Pollack 1938). Reports concerning blood cholesterol changes after epinephrine administration are very varied in different authors. Burger and Rosenthal (1934) reviewed the literature and their own studies indicate no changes. Concerning fat transportation and fat depots, in rats, epinephrine increases liver lipids and reduces adipose tissue lipids. (Clément & Schaeffer 1947). Perineal fats of the rat were shown to be specially decreased after epinephrine administration. (Clément & Schaeffer 1947). Daily intravenous administration of epinephrine to the rabbit, increased the plasma neutral fats and decreased the plasma cholesterol at the same time. The lipemia (which) caused this way was visible. (Dury & Moss 1954). These findings could be important concerning the arteriosclerotic effect on the vessels caused by epinephrine.

Experiments with Norepinephrine showed an increase of neutral fats of the rat's liver, but no changes in cholesterol or phospholipids. (Aujard 1953).

Wool (1953 & 1954), reports that fat mobilisation and storage in the liver was only possible when cortisone was added to adrenalectomized rats, or when epinephrine was administered to adrenodemedullated rats. This

demonstrates, that the adrenal cortex and adrenal medulla are important for the interaction of hormones. An example herefore can be shown with the experiments by which adrenalectomized animals could not maintain a normal amount of fat in adipose tissues, unless cortisone and ethionine-epinephrine was given.

Wertheimer, Shapiro (1948) and Deuel (1955) agree on the conclusion, that sympathetic innervation of adipose tissue is important for normal fat storage and transportation. The mechanism of action of epinephrine on fat transport is not well known. There is possibly a direct relationship to the glycogenolytic effect in the liver. Glucagon, which also depletes the liver glycogen is well known to stimulate the fat transport into the liver. If epinephrine was not as effective in this action, it may be mentioned, that glucagon depletes the liver of its glycogen for more prolonged periods of time than epinephrine, thus causing more opportunity for the transport of fat.

It is also presumed (Costa, Galaniso & Foá 1956), that epinephrine action may be mediated by the pituitary as well as by the adrenal glands.

Metabolism of Nitrogenous Organic Substances.

C. Protein Metabolism.

Blum (1901) observed, that in the so called "adrenal diabetes" no increased urinary nitrogen excretion was found. The same results were obtained after prolonged epinephrine infusion, (Samson & Jacobs 1932). In intact animals epinephrine induced urine flow, which regulates the urea excretion, has led to insignificant changes in protein catabolism, (Toth 1937). In undernourished or fasted animals urinary nitrogen excretion was increased by epinephrine, (Reid 1941, Patonisoi, Underhill 1906, Allan, Dickson & Markowitz 1924). Glucose or glucose and insulin administration reduced the action of epinephrine on protein catabolism, (Engel 1952,Reid 1941). It has been postulated, that fasting increases the negative nitrogen ballance caused by stress (Werner 1951). Protein catabolism was stimulated in rats by administration of large dosages of epinephrine.(Epinephrine stress) (Engel 1952, Noble & Toby 1948). The catabolic effectof epinephrine on protein was even more pronounced when trauma was super imposed (Noble & Toby 1948). Engel 1952, ascribes a definite rate to the adrenocortical hormones in epinephrine stress. In

his experience with adrenalectomized-nephrectomized rats a catabolic effect of epinephrine was observed only when the rats were pretreated with cortisone. Rose and Nelson (1956) report, that in their experiments with nephrectomized rats intravenous and intraportal epinephrine or glucagon increased urea production, when the rate of administration was adequately high in order to cause depletion of liver glycogen. These results lead to the assumption, that the depletion of liver-glycogen represents the required stress for the catabolic effect of epinephrine; but that the liver is not able to respond to this stress without the presence of cortical hormones. Cori (1931) confirmed the findings, that the effect of epinephrine on protein metabolism represents only a small part of the calorigenic effect and of the effect on gluconeogenesis. Numerous reports exist concerning proteinuria associated with epinephrine administration. (King & Baldwin 1955, Starr 1926, Toth 1937, Zueler 1901). The following hypoalbuminuria can not be considered as the only consequence of increased protein catabolism.

Epinephrine administration is also followed by a decreased amino acid nitrogen level in the blood. According to Luck and Spaulding (1928-29), this action

of epinephrine is not mediated by the pituitary-adrenal axis. The same effects have been observed by administration of levarterenol to hypophysectomized rats. The mechanism concerning the action of epinephrine on blood amino acids is not well understood at present. It may be mediated indirectly by the pancreas, since the same effects have been demonstrated after insulin or epinephrine administration.

It has also been reported, that insulin reduced the blood amino acid level in eviscerated and eviscerated-adrenalectomized rats, while epinephrine was not acting similarly in the same case. (Hussay and co-workers 1937). This can be explained either with the absence of insulin, or with lack of the viscera, which may have an effect on the action of epinephrine. Other factors may play a role.

In anesthetized animals, no hypoaminoacidemia response has been observed following administration of epinephrine, proving the fact, that anesthesia diminishes insulin secretion, which is required for this process.

Luck and co-workers (Davis & van Winkle 1934, Griffin & co-workers 1954) showed that the presence of epinephrine was necessary for the action of insulin on blood amino acids, since there was no hypoaminoacidemia

in adrenalectomized rats. According to Luck and Spaulding (1928-29) blood amino acids have been decreased, when insulin hypoglycemia was prevented by glucose administration. This confirms the reports of Ingle and co-workers (1955) according to which insulin is supposed to be the factor reducing blood amino acids in eviscerated and eviscerated-adrenal-ectomized rats. It can be concluded, that epinephrine causing hyperglycemia will stimulate a release of insulin which is followed by decrease in blood amino acids. (Russell 1955). Other epinephrine like substances have been used in several experiments (Sahyun 1933) like: norepinephrine, phenylephrine, ephedrine, metamphetamine, phenylethylalamine et-al.

Levarterenol was ineffective in normal animals but caused hypoaminoacidemia in hypophysectomized rats. (Griffin & co-workers 1928-29). But the reducing effect on blood proteins has been found only following epinephrine administration. (Bruinsk & Luck 1952).

1. Creatine Metabolism.

Creatine is normally formed in the liver and transported to the muscle, where it is assimilated and stored. In some pathological conditions like progressive muscular dystrophy and diabetes mellitus, the creatine excretion is increased. The cause of this finding is not well known. The excreted creatine derives either from the muscle stores or it is due to a lack of assimilation by the muscle.

Rose (1935) in his review of the literature mentions a relationship existing between muscle glycogenolysis and increased creatine excretion. Comsa (1948) states that there occurs marked creatinuria after administration of epinephrine, if the muscle glycogen is severely depleted. This is not the case, when enough muscle glycogen is present. After administration of epinephrine creatine excretion was increased in normal animals and in those pretreated with thyroid hormone. (Comsa 1948, 1950, Pflug 1938). Tissue creatine was increased, unchanged or slightly increased in the muscle. (Pataky & Pfeifer 1951, Koven, Fizzolato & Beard 1940). It was reduced in the heart and unchanged in the brain after epinephrine administration. (Ores & Abelin 1954).

According to Blise, Rubin & Gilbert (1951) an increase in creatine in human subjects has been observed

after epinephrine administration. This effect however, is more or less a measure for the effect of glomerulor filtration rate, rather than a sign of change in creatine-creatinine metabolism (Ellis 1958). Norepinephrine, phenylephrine and ephedrine did not show the same effect on creatine metabolism as epinephrine.

2. Uric Acid.

The effect of epinephrine upon uric acid metabolism was reviewed by Bishop & Talbot (1953) and by Chaikoff (1935). An increase of uric acid and allantoin has been observed in animals after administration of epinephrine in high dosages or as a result of insulin hypoglycemia. (Chaikoff 1935, Larson & Brewer 1936, Miller & Kuyper 1938).

It has been suggested, that the effect of epinephrine on uric acid may be activated by pituitary and the adrenals. This has been supported by the fact, that large dosages of epinephrine are required for the effect to take place, that adrenocortical hormones have the same action, that there was no effect of epinephrine in Addisonian disease and that adrenalectomized animals with small rests of the adrenals left, show similar effect. (Bishop & Talbot 1953, Bliss, Rubin & Gilbert 1951, Larson & Brewer 1936).

D. Influence of Catechol Amines on Oxygen Metabolism.

1. Calorigenic Effect.

Belawenetz had already discovered in 1903 that epinephrine increased oxygen consumption in almost all species. He stated however, that the effect of epinephrine on oxygen consumption depended on the dosage administered. In his experience Belawenetz observed, that large dosages of epinephrine decreased oxygen consumption and body temperature. Since these findings have also been confirmed by other investigators, (Trendelenburg 1924 & 1934, Essex 1952) it was postulated, that large epinephrine administration first causes a fall in oxygen consumption due to respiratory depression, than an increase, as a consequence of interference with gaseous exchange and pulmonary edema. The effect of epinephrine on pulmonary blood flow was known since the experiments of Delaunois (1945) performed on dogs.

Boothby and Sandiford reviewed this subject in 1923 and they postulated, that a major part of extra oxygen consumption must be attributed to fat catabolism. The excess oxygen metabolism caused by epinephrine is not limited to a single organ. Investigations on the liver, the heart, the muscle and the sympathetic nervous system have been performed. A

transient heat increase was demonstrated on the liver in vivo, after a relative high dosage of epinephrine. (Jitariu, Koch and Otto 1941). This finding was considered as a secondary effect to epinephrine in the liver, since the same experiments with intraportal epinephrine administration have been less effective. Experiments on hepatectomized frogs indicate, that the liver is not essential for the calorigenic effect. (Cori and Buchwold 1930-31). Soskins (1927) found that there was no calorigenic effect of epinephrine in eviscerated or hepatectomized animals. Drury and his co-workers (1955) noted a marked hyperlacticacidemia in eviscerated animals. This was explained by Lundholm (1949) who has found hyperlacticacidemia to follow epinephrine administration and postulated, that the high lactate of hepatectomized animals possibly masks the effect of epinephrine on oxygen consumption. Intravenous infusion of epinephrine increased cerebral metabolism,(King and co-workers 1952). This however was not observed after intramuscular administration. (Sensenbach 1953). Several authors found that other pressor amines had no calorigenic effect. (Moyer, Morris and Snyde 1954). The calorigenic effect of epinephrine on the heart is rather controversial. Green, Euler and Moe (1953) have found, that under some conditions epinephrine increased oxygen

consumption more than work. A reduction in oxygen consumption was found in heart-lung preparations after administration of N-phenyl-N-isobutyl-nor-p-sympathol, N-phenyl-N-butyl-norepinephrine, as well as after epinephrine.(Gollwitzer-Meyer and Jungmann 1952). The effect of epinephrine however is controlled by reflectory activity on the heart. The effect was less marked in innervated lung-muscle preparation and in the heart in situ, than in denervated lung-heart preparations.

Epinephrine or sympathetic stimulation did not increase oxygen consumption of a resting heart. Lee (1953) demonstrated, the difference between the action of glycosides and epinephrine on the heart muscle. Ouabain improved the muscle contraction without changing oxygen metabolism, while epinephrine increased oxygen consumption with increase of contracting power. There is evidence that a transient calorigenic increase after intraarterial administration of epinephrine is present in skeletal muscle.(Bucherl and Schwab 1952), Kleinsorgand and Schmien 1952). This effect could be observed as an initial reaction to the hormone, as stated by Mertens and Rein (1938) and to be followed by adaptation and normalization. The effect in intact animals has been attributed to reoxygenation of "pooled" blood. Since Lundholm (1949) showed, that the calorigenic

effect of epinephrine was related to serum lactate levels, a more prolonged administration of epinephrine could clarify the situation.

In smooth muscle De Meio (1941) found, that there was an increased oxygen consumption in reproductive organs of the rabbit, but not in liver or diaphragm. Bulbring (1953) observed an increased oxygen metabolism in guinea pigs intestine under stress, but a decrease under resting conditions. These findings were confirmed by Rao and Singh (1940) in smooth muscle preparations of the frog depressed by epinephrine. Strand and Gordon (1953) found, that in intact, adrenalectomized or hypophysectomized rats a depressed respiration was present after epinephrine administration in the following organs: the lymph nodes, the thymus, the spleen, the liver and the muscle. There is no explanation available for these findings.

2. Mechanism of the Calorigenic Effect.

In his recent review of the calorigenic action of epinephrine, Lundholm (1949) concluded, that the mechanism of increased oxygen metabolism was due to hyperlacticacidemia with increased tissue metabolism. Griffith (1951) attributes the calorigenic action of epinephrine to many factors, which include increased body temperature, increased activity of cardiac and skeletal muscle, plethora of glucose and lactic acid and increased cellular metabolism. Recent analysis refers to earlier work of Green and Richter (1937), which attributed several effects of epinephrine to its decomposition product, adrenochrome, which stimulates some of the enzyme systems involved, while others are inhibited.(Radsma & Golterman 1954). According to some findings diminished oxygen consumption occured after epinephrine administration. (Griffith 1951).

This could be explained by the fact, that epinephrine causes vasoconstriction to the skin vessels. Thus increase in body temperature and delayed increase in oxygen use. This mechanism is appliable to low dosages of epinephrine, while after higher dosages hypothermia results. (Trendelenburg 1924,Essex 1952). Whitcher and Griffith (1949) found, that in intact cats the temperature increased in response to

epinephrine; in skinned cats the temperature decreased and the calorigenic effect of epinephrine was smaller. From these experiments it may be concluded, that constriction of skin vessels by epinephrine conserves the body heat and increases the calorigenic effect. The skin itself plays a minor role in this mechanism. Isoproterenol increases oxygen consumption and body temperature in the rat. (Waterman 1949). It was noted in these experiments, that the skin was rather reddish than blanching, which could be explained in such a way, that isoproterenol was lowering the blood pressure sufficiently, to produce low blood flow in the skin and still influence its calorigenic effect. Increased muscular activity may be of considerable importance in the calorigenic action. The missing effect of epinephrine in anesthetised animals may be explained that way. Lundholm (1949) found that anesthetic agents which reduce the calorigenic action, also reduce the blood lactate in response to epinephrine. Extirpation of the sympathetic nervous system did not reduce the calorigenic effect of epinephrine. (Lee & co-workers 1933).

Epinephrine hyperglycemia does not coincide with the calorigenic effect,(Boothby & Sandiford 1923). This was shown by Lombroso (1937). Adrenergic block-

-ing agents do not prevent epinephrine hypermetabolism, but they do prevent hyperglycemia.

Isoproterenol for example, was found to increase the metabolism of the rat (Waterman 1949) but not the blood sugar (Ellis & Anderson 1951).

In comparative studies by Whelan and Young (1953) they found that both catechol amines stimulated respiration, but only epinephrine increased oxygen consumption. Levarterenol was found to have the same effect as epinephrine. (Vleeschouwer & Smythe). Bearn (19) found an increase in blood lactate only 10 minutes after epinephrine infusion.

Lundholm and Mohme (1949) showed, that ergotamine administered to guinea pigs 20 minutes previous to epinephrine injection, inhibits the calorigenic effect. Other authors stated, that ergotamine prevented the calorigenic effect only when small dosages of epinephrine were given. (Lopes & co-workers 1954, Lundholm & Mohme 1949, Rothlin & Cerletti 1952). The evidence that ergot alkoloids can prevent the calorigenic effect and the hyperlacticacidemia following epinephrine, prove the hypothesis that high blood lactate causes the calorigenic effect of epinephrine. (Lundholm & Mohme 1949, Goldblatt 1933).

5. Effects of Catechol Amines on the Central Nervous System.

Transmission of peripheral synapses. Marrazzi(1939) has demonstrated , that epinephrine exercised a depressant action upon transmission in the superior cervical sympathetic ganglion of the anesthetized cat. A dose of 10-250 mcrg. of epinephrine intravenously caused a diminution or a disappearance of the postsynaptic spike when the presynaptic nerve was electrically stimulated. Marrazzi(1939) postulated, that this inhibiting effect of epinephrine on sympathetic ganglia was a direct action, probably necessary for the prevention of excessive sympathetic action in certain conditions. These experiments have been extended to sympathetic and parasympathetic ganglia in other locations and it could be demonstrated, that the action of epinephrine was localized at the synapse itself.(Marrazzi 1952, Tum Suden & Marrazzi 1951, Tum Suden & co-workers 1951).

Lundberg(1952) performed similar experiments and found that as little as 1mcrg. of epinephrine administered intravenously produced a depression of neurotransmission. In experiments with Norepinephrine it could be demonstrated that only one-fourth or one-third of the effect produced by epinephrine was attained. According to Lundberg(1952) there was no depolarization or hyperpolarization present. He has postulated, that

the ganglionic blocking action of epinephrine was comparable to the action of curare. Matthews(1956) observed a depression of transmission at peripheral synapses with 0,3-0,7 mcrg/kg. of epinephrine. Norepinephrine was found to be much less effective. Matthews(1956) also demonstrated, that the depressant effect of epinephrine was affecting only two of the three sets of postsynaptic fibres leaving the ganglion, namely, the fibres presumably destined for the nictitating membrane and blood vessels. None of the above investigators observed any augmentation of postsynaptic neurotransmission by epinephrine.

Bulbring and Burn(1942) used in their experiments preparations of the hind limbs and the abdominal sympathetic ganglia. When small amounts of epinephrine were added to the perfusion, an increase in vasoconstriction of the limb was observed with electric stimulation. Larger dosages however, seemed to cause a depressant effect. The same results have been attained when the superior cervical sympathetic ganglion was perfused and very small amounts of epinephrine were added. With larger dosages of epinephrine in the perfusion, the response of the nictitating membrane was depressed. Malmejac(1955) confirmed the observations of the other authors, that small doses of epinephrine

(0,5-4 mcrg./kg.,per minute) seem to enhance the response of sympathetic ganglia, while larger doses (12-15 mcrg./kg.,per minute) decrease the response. Kouzett(1950) observed an augmentation of the response to acetylcholin injection, when in very small doses epinephrine was added. When comparing this with other catechol amines and their derivatives, Kouzett concluded, that the ganglionic response to these drugs resembled those of the beta adrenotropic receptors of Alquist (1948). Kouzett also observed, that sympathicolytic drugs did not abolish the augmenting effect of epinephrine on acetylcholine.

Matthews(1956) reported, that Dibenzyline did not block the effect of epinephrine and norepinephrine in his experiments.

Kewitz and Reinert(1952 & 1954) compared the effect of epinephrine on the perfused ganglia, following acetylcholine injection and preganglionic nerve stimulation. It was observed that, as a rule, epinephrine augments acetylcholine-induced response but diminishes the response to nerve stimulation. Kewitz(1955) doubted then, that acetylcholine be the physiological transmitter substance at this special synapse. Most probably the results will depend on whether an electric stimulus of the preganglionic nerve has been used, or whether acetylcholine was

injected into the perfusate. It is also important, whether the nictitating membrane, the adrenal medulla or the vasoconstrictor nerves have been used as indicators. It may still be questioned, whether the effects of epinephrine and norepinephrine on the peripheral ganglia are revelant, since the preganglionic nerve fibres are presumed to be cholinergic. Attention has been called to the fact, that there were chromaffin cells found in sympathetic and parasympathetic ganglia and that these cells possibly released adrenergic substances into the synaptic region of the ganglion. (Bulbring 1944, Tum Suden & co-workers 1951). The release of an epinephrine-like substance into the ganglion perfusate following stimulation has been reported. However the purpose of this mechanism is not well known yet.

The possibility that C.A. act on the brain indirectly, by constriction of cerebral vessels, has been considered since the early 20th century. There are reports according to which epinephrine dilates cerebral vessels, constricts them, or performs both effects consecutively. Forbes (1933) summarising earlier reports and evaluating his own experiments, observed a definite effect of epinephrine on cortical-cerebral vessels. Epinephrine, locally applied to

cortical vessels, produced a constriction of larger vessels only, while intravenous administration of 1-100mrcg. of epinephrine caused as a rule vasodilatation. Intracarotid administration of epinephrine produces vasodilatation followed by vasoconstriction, after the blood pressure returned to normal levels. Fog (1939) used a similar technique and found that epinephrine, in very low concentrations, constricted the brain arteries, while the arterioles remained unaffected. Experiments employing intravenous application of epinephrine have demonstrated, that this effect was depending entirely on the rise in blood pressure. Sympathomimetic and parasympathomimetic drugs injected into pial arteries, produced constriction of larger arteries only.(Lunn & Fog 1939). Schmitt (1956) studied the effect of epinephrine and norepinephrine upon blood volume in the brain and demonstrated, that 50 mcrg. of both hormones produced vasoconstriction, especially following epinephrine administration. In a shock state both hormones produced vasodilatation. Bovet & Carpi (1958) demonstrated, that the vasoconstricting effect of epinephrine on the brain disappears during deep anesthesia, especially after administration of barbiturates. Respiratory acidosis, extensive surgery and shock are also counteracting epinephrine induced cerebral vaso-

constriction. These findings were interpreted as a protective mechanism in animals in a poor general condition. King (1952) reported an increase in cerebral blood flow in men with pressor dosages of 22-73 mcrg./per min. of epinephrine, and a decrease with norepinephrine. Sensenbach (1953) who has administered the hormone intramuscularly in dosages of 0,6-1,0 mg. found a decrease of blood flow with norepinephrine, but no changes with epinephrine. More recently Ingvar and Soderberg (1956) demonstrated clearly, that norepinephrine, administreed intracarotid, caused an initial vasoconstriction of cerebral blood flow, followed by vasodilatation, while epinephrine caused vasodilatation only. It has been postulated, that epinephrine and norepinephrine produced changes in EEG and that epinephrine only caused an increase in oxygen consumption in the brain. Epinephrine and norepinephrine do not appear to act equally on all parts of the brain, as it was demonstrated by Schmitt (1934) who claimed, that these drugs have almost no effect on the medulla vessels, but a mild and persistent constricting effect on the hypothalamus. Studies by Watts & Pool (1957), by Jones & Blake (1958), by Leimdorfer (1957) and others have shown, that injected epinephrine and norepinephrine are removed from

circulation whithin few minutes, mainly by the liver and the kidneys. Whithin 20 hours all of the hormone is excreted into the urine. In the cerebral fluid, these hormones are stable in solution. There is evidence, to suggest, that epinephrine and norepinephrine penetrate the blood-brain barrier remaining there longer than in the body. According to some authors, the effects observed, the action and excretion of C.A. are different in various parts of the brain, depending on whether the administration was carried out intrathecally, intracisternally, into the ventricles or into the spinal cord.

The effects of epinephrine and norepinephrine on the brain have also been observed by demonstration of an indirect or reflectory mechanism of action, through the carotid and aortic baro-receptors. These results were obtained in connection with studies of a direct depressing effect on the hormones upon the vasomotor centre. Heymans and his co-workers(1953) observed, that any drug, raising the blood pressure, stimulated the pressure-sensitive elements in the carotid sinus and the aortic arch, which send impulses to the vasomotor centre. These reflectory impulses inhibite the action of the vasomotor centre and thus restore the blood pressure back to normal levels. Epinephrine was shown to act in a similar way, inducing reflex-vasodilatation

and bradycardia with the rise of blood pressure. Similar effects of both hormones have been demonstrated with local application to the carotid sinus. (Heyman 1955). They generally tend to diminish the vasomotor action. In the light of present knowledge, it can only be stated, that epinephrine and norepinephrine increase the activity of the carotid and aortic baroreceptors, by direct stimulation and sensitisation and by rise of the blood pressure. The consequence of this action is an inhibition of the vasomotor centre and, probably, other brain stem mechanisms are inhibited as well, including the respiratory centre and the reticular activating system. Possibly direct inhibiting effects of the vasomotor centre are brought upon by other intracerebral chemoreceptors. Whether anesthetized animals and a relative unphysiologically high dosage of the drugs play an important role, has not been demonstrated decesively.

Epinephrine stupor, deep analgesia and sleepiness were proven to be the most marked effects of larger dosages in experimental animals and man, demonstrated by many authors since the beginning of this century. (Donitz 1903, Zeigan 1904, Weber 1904, Ivy1947). The administration of the hormone was either intralumbar, intravenous, intracarotid or intracisternal and intraventricular. Invariably deep analgesia and sleepiness followed a short period of salivation or restlessness. In all cases however consciousness was maintained. The dosages used were ranging between 0,2-1,0 mgm. In cats 5 mgm. of epinephrine were found to be the lethal dosage. 100 mcrg./Kg. of epinephrine given to unanesthetized dogs produced initially excitement, vomiting, stupor and spasticity but was followed by a deep analgesia of 60-90 minutes duration, in which time an abdominal operation could be performed on the animal. A marked elevation of the pain threshold has been demonstrated by the tooth pulp method. Leimdorfer (1947) found an alteration of blood sugar following intracisternal epinephrine injection. This latter type of administration of epinephrine seems to be more effective on analgesia and sleep even in lower dosages. No changes in the EEG were observed. Leimdorfer (1950) compared the intracisternal effects of epinephrine, norepinephrine, isoproterenol and

butanephrine and they all produced stupor or sleepiness. Neosynephrine, ephedrine,amphetamine and tuamine produced great excitement. The injection of 20-80 mgm. of epinephrine or norepinephrine into the lateral ventricle produced a light anethesia comparative to pentobarbital. EEG changes were insignificant during this time. Respiration was found to be deep and accellerated. Segmental reflexes (like extensor stretch reflexes, limb withdrawal, corneal, conjunctival etc.) have all been maintained. Analgesia and stupor were always present; there was no analgesia in a completely alert animal. (Rothballer & Sharpless unpublished observations.) Ivy (1944)could demonstrate analgesia in human beings without accompanying stupor. This observation found the bases for the attempts to use epinephrine and congeners as an anesthetic. Gross (1948) used epinephrine in patients affected with leprosy and succeded in production of a marked elevation of the pain threshold in these subjects. Wolff (1940) proved that epinephrine abolished the effect of morphine, but that on the other hand it was very effective in prolonging anesthesia with barbiturates, paraldehyde, chloralose and ethanol.

CHAPTER VI

MATERIAL AND METHODS

1. Animals

All rats used in this experiment were of the Royal Victoria Hospital-Hooded strain and were bred in the laboratories of the department. Only male rats weighing between 150-280g. were used, to avoid the possibility of response due to sex differences. As outlined later under description of collection of gastric secretion, animals were uniformly fasted for 24 hours prior to the experimental procedure. However, a group of non-fasted animals were also prepared in order to study the effects of fasting on C.A. (Catechol Amines)

2. Method of collection of gastric secretion

a) Anesthesia and the operating room set-up

All operating procedures were carried out in the small-animals operating room using clean surgical instruments which have been kept in Hibitane disinfectant solution. The sutures used in the procedure were those of 00-silk thread for the intra-abdominal part of the operation and 000-silk thread for closure of the abdominal cavity. The animals were anesthetized by intraperitoneal injection of Nembutal in the dosage of 0.1cc. (0.6 grain) per 100gr. of body weight.

The vast majority of animals tolerated the

anesthetic well and remained anesthetized for periods ranging from 5-45 minutes, varying slightly in some of the animals. After the administration of the anesthetic the animals were placed on an operating board with the front teeth hooked on a flange and the extremities attached by rubber bands. The abdomen was then shaved and the skin sterilized by application of Hibitane solution. Approximately 5% of animals died due to anesthesia.

b) Operative procedure

The abdominal organs were exposed through a median laporotomy of about 1-1½". Blunt forceps were used for the exteriorating of the stomach. A needle was then inserted into the esophagus and the stomach was irrigated with physiological saline to remove food remnants. The cardia and the pylorus were then ligated with OO-silk. Special precaution was taken, so that the vascular supply was not damaged or included in the ligature. In cases where the vessels were injured or ligated a hemorrhagic gastritis resulted and the admixture of blood necessitated the exclusion of the gastric juice from further use. The cardia ligature was placed beneath the vagus nerves so that no pressure nerve damage was involved.

After 24 hours the animals were sacrificed and the secretion accumulated in the stomach pouch was emptied into a beaker by incising the forestomach. The collection procedure was best carried out after the stomach was removed by transection

above the cardia and below the pyloric ligature. The secretion collected this way probably included some transudate from the distended gastric wall. The amount however, was insignificant. The volume of secretion collected, was used for estimation of pH, hydrochloric acid, sodium potassium and chlorides. The pH was determined on Beckman p.H. meter. Free HCl was estimated by titration with 0.1 N sodium hydroxide using Topfer's reagent indicator. Chloride was measured by the method described by Schales and Schales. Sodium and potassium were determined using a flame photometer.

3. Method of collection of urine for estimation of C.A.

a) Experimental set-up

The animals in which the cardia and pylorus were tied were all kept separately in specially designed metabolic cages. As mentioned previously, the animals were fasting for the preceding 24 hours therefore, no difficulties due to passages of food particles into the urine collection containers were encountered, except in the group of animals in which the food was not withdrawn to compare the C.A. values with those of fasting animals used in all experiments. In the non-fasted group on several occasions food particles have been observed to pass through the mesh wire at the bottom of the cage into the urine-collection containers. The metabolic cages were modified in such a way that an additional fine gauge wire sheet was placed in each cage to prevent the passage of feces into the urine-collection containers. This was very effective and on no occasion were feces observed to pass into the containers, except

for some animals to which Reserpine and Reserpine and Histamine in combination had been administered. In this group fecal matter was observed in the urine-collection containers in approximately 3-5% of the cases. The collection flasks were attached by means of a perforated rubber stopper to the funnel at the bottom of the metabolic cage, so that no loss of urine was encountered.

b) Method of collection

The rats were placed separately in the metabolic cages and the urine was collected in a graduated flask for the following 24 hours. The content of the flasks of the whole group was then pooled and a solution of 50% HCl acid plus a few drops of toluene were added to the urine. The container was then covered with wax paper and placed in 4°C. temperature until the analysis was carried out, which was usually done 24 hours later.

4. Methods of estimation of C.A. in the urine

a) Method

The method used for C.A. estimation in our experiments originates from U.S. von Euler which for our studies was modified from the above mentioned method as proposed by Dr. R. Hobkirk of the Montreal General Hospital.

b)

The following is an excerpt from Dr. Hobkirks description

"PRINCIPLE

Urinary catecholamines stabilized with ascorbic acid, are adsorbed on alumina columns at an alkaline pH and are partially purified by washing with sodium acetate solution and water. Elution is easily brought about using 0.2M acetic acid. Adrenaline and noradrenaline are both oxidized by a variety of oxidants (in this case iodine) to the corresponding chromes at pH 6.0, whereas at pH 3.5 only adrenalin is oxidized (4). By performing oxidation at both pH values a differential estimation of the two catecholamines is made. After destruction of excess oxidant (in this case with $Ma_2S_2O_3$) the chromes are isomerized to the corresponding lutins (3, 5, 6, trihydroxy-indoles) with alkali (5). These lutins fluoresce when irradiated by light of a suitable wave length and can be measured fluorimetrically when stabilized with ascorbic acid.

REAGENTS

All solutions for this method are prepared using double glass distilled water. Double distilled water is also implied wherever water is mentioned elsewhere in the procedure.

1. Versene solution (10%)

50 g. of the disodium salt of ethylenediamine tetracetic acid are dissolved with stirring in 500 ml. of water. This solution is kept in a glass bottle fitted with a burette.

2. Ascorbic acid solution (2%)

200 mg. of ascorbic acid (Nutritional Biochemicals for

investigational use only) are dissolved in 10 ml. of water. This solution is prepared immediatly before use.

3. Sodium hydroxide solution (4%)

20 g. of sodium hydroxide pellets are dissolved in 500 ml. of water and kept in a tightly stoppered bottle. For use, this solution is contained in a small bottle fitted with a medicine dropper.

4. Sodium hydroxide solution (20%)

100 g. of sodium hydroxide pellets are dissolved in 500 ml. of water and kept in a tightly stoppered bottle.

5. Alumina for chromatographic analysis (Woelm; almost neutral; activity grade I.)

6. 0.2 Molar sodium acetate solution (pH 8.4)

16.40 g. of anhydrous sodium acetate or 28.20 g. of the trihydrate CH_2COONa, $3H_2O$, are dissolved in water and made up to 1 litre with water in a volumetric flask. A very few drops of 0.1 N NaOH are added to bring the pH to 8.4 using a glass electrode.

7. 0.2 Molar acetic acid solution

Measure out 12 ml. of glacial acetic acid in a graduated cylinder. Make up to 1 litre with water in a volumetric flask and mix thoroughly. Standardize with 0.1 N NaOH using phenolphthalein as indicator. Suppose 10 ml. of the acetic acid solution are neutralized by V ml. of 0.1 N NaOH. Then, normality of the acetic acid is $\frac{V \times 0.1}{10}$. To adjust the normality to 0.2 take a convenient volume of the acid (V ml.)

and dilute it with V (0.05 x T-1) ml. of water.

8. Molar acetic acid solution

By means of a graduated cylinder measure 60 ml. of glacial acetic acid into a 1 litre volumetric flask; make up to the mark and mix thoroughly. This need not be standardized.

9. Molar sodium acetate solution

82.0 g. of anhydrous sodium acetate or 136.0 g. of the trihydrate, $CH_3.COONa, 3H_2O$, are dissolved in water and made up to 1 litre with water in a flask.

10. Molar acetate buffer pH 3.5

Mix 190 ml. of reagent 8 with 10 ml. of reagent 9 and mix. Check the pH with a glass electrode and adjust if necessary with solutions 8 or 9 as required. Store at $4^{\circ}C$.

11. Molar acetate buffer pH 6.0

Mix 30 ml. of reagent 8 with 600 ml. of reagent 9 and mix. Check the pH and adjust if necessary with solutions 8 and 9 as required.

12. Adrenaline stock solution (100 mg. of free adrenaline per ml.)

Weigh out accurately 18.2 mg. of adrenaline bitartrate. Dissolve in 0.2 M acetic acid and make up to 100 ml. with this acetic acid in a 100 ml. flask. This solution is stable for 6 weeks if kept at a temperature of approximately $4^{\circ}C$.

13. Adrenaline working solution (1 mcrg. of free adrenaline per ml.)

1.0 ml. of reagent 12 is pipetted into a 100 ml. volumetric flask and made up to the mark with water. This solution is prepared fresh daily.

14. Noradrenaline stock solution (100 mcrg. of free noradrenaline per ml.)

12.2 mg. of noradrenaline hydrochloride are accurately weighed out, dissolved in 0.2 M acetic acid, and the solution made up to 100 ml. with the acid in a volumetric flask. This solution is stable for 6 weeks at 4°C.

15. Noradrenaline working solution (1 mcrg. of free noradrenaline per ml.)

1.0 ml. of solution 14 is pipetted into a 100 ml. volumetric flask and made up to the mark with water. This solution is prepared fresh daily.

16. 0.004 Normal iodine solution

0.2 ml. of the 1N commercial standard iodine solution is measured by graduated pipette into a 50 ml. measuring cylinder and made up to 50 ml. with water. This is prepared fresh daily.

17. 0.005 Normal thiosulphate solution

0.2 ml. of the 1N commercial standard sodium thiosulphate solution is measured by graduated pipette into a 50 ml. measuring cylinder and made up to 40 ml. with water. This is prepared fresh daily.

18. Alkali-ascorbic acid solution (reagent 2) is mixed with 18 ml. of 20% NaOH. This solution is very unstable and must be prepared immediately before use.

19. Quinine sulphate solution (0.25%)

0.5 g. of quinine sulphate is dissolved in 200 ml. of approx. 0.1N sulphuric acid. This solution is stable indefinitely at room temperature.

PROCEDURE

Preparation of chromatographic columns

These columns of are glass, 18 cm. long and 5-6 mm. internal diameter. Each has a bulb reservoir of capacity 50 ml. at the top and a ground glass stopcock at the lower end. There is a constriction in the tube just above the stopcock. A small plug of glass wool is inserted and packed against the constriction by means of a glass rod. 0.7 g. of alumina is measured out in a 15 ml. graduated centrifuge tube, 0.7 g. occupying 0.7 ml. in volume. This alumina is then introduced into the empty reservoir of the dry column, the column being held in an almost horizontal position with stopcock fully open. The alumina is then carefully washed into the column by means of about 5 ml. of 0.2 M sodium acetate, pH 8.4. At this stage the column is held at an angle of about 45°. When all the alumina is washed down, the column is clamped vertically in position. The surface of the alumina is allowed to settle under gravity with frequent sharp tapping of the column and a small plug of glass wool is placed on top to protect the surface. When the level of the sodium acetate solution has just reached the top of the alumina (6) the stopcock is closed and the column is ready for use.

Preparation of urine for chromatography

The 24 hour urine specimen is diluted with water, to 2000 ml. (7), (in our case to 100 - 150 - 200 ml.). Duplicate 25 ml. aliquots of the filtered urine (8) are measured by graduated pipette into each of two 100 ml. beakers. To each is then

added 1 ml. of 10% versene (9) and 1 ml. of 2% ascorbic acid (10). Each beaker is then successively placed on an electric magnetic stirrer, a magnet is placed in the solution, and the combined electrode of a pH meter previously standardised with known buffers is lowered into the solution. Careful stirring is commenced with the dropwise addition of 4% NaOH until the pH has reached a value of 8.4 (11). Stirring is stopped, the pH meter is switched off, the magnet is removed with forceps and rinsed into the solution with a jet of water from a wash bottle and the urine is transferred immediately to the column, care being taken to disturb the surface of the alumina as little as possible. The beaker is rinsed twice with 1 ml. volumes of 0.2 M sodium acetate (pH 8.4), the washings being added to the column. The beaker is then used for collection of the solution from the column.

CHROMATOGRAPHY

The stopcocks of the columns are opened and the rate of flow of the urine solution adjusted so that the entire volume passes through in 10-20 minutes (12). The stopcocks are closed when the solution level has just reached the alumina (8). The columns are then washed by passing first 5 ml. of 0.2 M sodium acetate (pH 8.4) and then 5 ml. of water (13). The rate of flow of these should be about the same as for the urine (12). The beakers, containing the spent urine and the washings, are removed and replaced by Quickfit B19 tubes. Exactly 5 ml. of 0. 2 M acetic acid are measured by pipette into the column, the

stopcock opened to give the above flow rate (14) and the eluate collected in the tubes. This is followed by exactly 5 ml. of water under the same circumstances (14). In each of these stops stopcocks are closed when the liquid level has just reached the alumina (8). The tubes containing the 10 ml. eluate are stoppered and, if not used at once for analysis, stored at about 4°C. (15).

OXIDATION OF THE CATECHOLAMINES

From each chromatographic eluate duplicate 1 ml. volumes are measured into each of two B19 stoppered tubes. These are used for oxidation at pH 3.5 and 6.0 respectively and are set up together with appropriate blanks and standards as follows (16).

Tube	Chromatographic eluate (ml.)	Adrenaline working sol. (ml.)	Noradrenaline working soln. (ml.)	Water (ml.)	M Acetate buffer pH 3.5 (ml.)	M Acetate buffer pH 6.0 (ml.)	
	1 ml. grad. pipette	0.2 ml. grad. pipette	0.2 ml. grad. pipette	burette	burette	burette	
1. Reagent blank	-	-	-	3	6	-	1
2. Reagent blank	-	-	-	3	-	6	1
3. Unknown	3	-	-	-	6	-	6
4. Unknown	3	-	-	-	-	6	6
5. Standard	-	0.3	-	2.7	6	-	1
6. Standard	-	0.3	-	2.7	6	-	1
7. Standard	-	0.3	-	2.7	-	6	1
8. Standard	-	0.3	-	2.7	-	6	1
9. Standard	-	-	0.6	2.4	-	6	1
10. Standard	-	-	0.6	2.4	-	6	1

By means of an automatic pipette, 0.9 ml. of 0.004 N iodine is added to each tube, with thorough mixing by shaking. Exactly 3 min. later 0.9 ml. of 0.005 N $Na_2S_2O_3$ is added from an automatic pipette to destroy excess iodine. Thorough mixing is again ensured. 0.3 ml. of alkaline-ascorbic acid solution is then immediately added (except blanks), with shaking from another automatic pipette. The tubes are stoppered and the contents are ready for reading.

FLUORIMETRY

The Coleman model 12c electronic photofluorometer is used with Corning filters 122 - 224 in the primary position (17) and Corning filter 14 - 212 in the secondary position (18). This instrument should be switched on 10 minutes prior to the commencement of readings (19). The instrument and galvanometer plugs are inserted and the main switch on the instrument is turned on. The phototube is switched on just before the readings are made. Special pyrex tubes (3 ml. capacity) are used for the reading. One of these is filled with quinine sulphate solution (20) and this tube is inserted into the 'standard' position of the instrument. Into the 'blank' position is placed a tube containing the reagent blank treated at pH 3.5 and in the 'Sample' position are put seccessively each of the solutions (unknown and catecholamine standards) oxidised at pH 3.5. After adjusting the galvanometer needle to zero using the dark current controls, the standard is placed in the light path, the shutter is raised, and the sensitivity knobs are adjusted to

read 100 mm. on the galvanometer. All of the solutions oxidised at pH 3.5 including adrenaline standards, are then read, ascertaining that the standard quinine sulphate reads 100 mm. (21) and reading the blank on each occasion (22). The pH 3.5 blank is then replaced by the pH 6.0 blank (23) and all of the solutions oxidised at this pH are read as above, including the adrenaline and noradrenaline standards.

CALCULATIONS

See page number 107 for the formula for calculations of catechol amines.

FORMULA

For the calculation of A. and N.A.

Adrenaline: $$x = \left(\frac{R^{u}_{3.5} \times 10}{3 \times S^{A}_{3.5}} \right) \frac{V}{25}$$

Noradrenaline:

$$y = \frac{R^{u}_{6.0} \times 10}{3 \times \frac{S^{NA}_{6}}{2}} - \frac{R^{u}_{3.5} \times 10 \times S^{A}_{6}}{3 \times S^{A}_{3.5} \times S^{NA}_{6}} \frac{V}{25}$$

$R^{u}_{3.5}$ = Reading of "Unknown" at pH 3.5

R^{u}_{6} = " " " at pH 6.0

$S^{A}_{3.5}$ = Standard for Adrenaline at pH 3.5

$S^{A}_{6.0}$ = " " " at pH 6.0

S^{NA}_{6} = Standard for Noradrenaline at pH 6.0

V = Volume of urine specimen diluted with water to 1,000 or 2,000 ml.
In rats we diluted to 100 - 150 or 200 ml.

SPECIFICITY

This method is quite specific for urinary adrenaline and noradrenalin. Few of the other related substances found in urine will interfere since a hydroxyl group on the Beta-carbon atom (adjacent to the ring) and a hydrogen atom on the Alpha-carbon atom are necessary for the reaction. Few compounds of of such a type are excreted in any considerable amount (29).

NOTES

1. The surface of the alumina must never be allowed to become dry since this introduces air pockets into the column which interfere with the chromatography.

2. This dilution ensures a reasonable rate of flow through the columns. If 24 hour volume is greater than 2,000 ml. dilution should be made to the nearest 100 ml.

3. Filtration is not necessary for every urine specimen but since it is better to filter cloudy urines this treatment may conveniently be applied in every case. Filtration also assists in speeding up flow through column.

4. Versene binds heavy metal in the urine and has been used by Weil-Malherbe and others as a means of presenting precipitation of phosphates etc. When the urine pH is adjusted on the alkaline side. This is of particular significance where columns are used for adsorption since such precipitates considerably reduce the rate of flow.

5. Ascorbic acid protects against later alkaline oxidation of the catecholamines.

6. A pH value between 8.3 and 8.5 is satisfactory. This is the optimal pH range for adsorption of adrenaline and noradrenaline on alumina.

7. It is essential that the catecholamines are in contact with the alkaline solution for as short a period as possible.

8. Sodium acetate and water remove a great deal of contaminating material from the column, thus improving the final measurements of the catecholamines.

9. The water serves to wash out the acetic acid remaining in the body of the column. This acid may contain a small amount of the catecholamines.

10. The eluates may be stored overnight at this temperature since the catecholamines are quite stable at this pH.

11. The table is drawn up for an individual urinary estimation. For duplicate analysis on one urine specimen a further two tubes would be necessary - twelve in all.

12. This enables the test solutions to be irradiated with light of wave-length 436 mmcr.

13. This secondary filter allows light of about 520 mmcr. to pass through to the photo-cell.

14. It is convenient to switch the instrument on just before iodine oxidation is commenced.

15. Quinine sulphate is strongly fluorescent in acid solution and this fluorescence is stable. Thus is useful as a standard solution to set the galvanometer scale.

16. Small fluctuations of the galvanometer needle occur rendering

it adviseable to check the standard setting before each reading is made.

17. It will be found on occasion that this reagent blank tends to give increased reading with time and it is therefore necessary to read it with each sample.

18. It should be understood that these values correspond only to the 'free catecholamines' excreted in the urine. There is, apparently, a further fraction excreted combined with glucuronic acid and perhaps sulphuric acid, which is not measured by the present method.

19. The values given represent catecholamines recoveries of the following order:

Adrenaline, mean recovery 92% (S.D.12)

Noradrenaline, mean recovery 90% (S.D.14)

Figures are not corrected for such losses.

The reproducibility of the method indicates duplicate analysis at normal catecholamine levels to differ by an average of 13% for adrenaline and 11% for noradrenaline."

5. Administration of compounds and division of experimental groups.

In the course of experiments several groups of animals were studied using various agents which either affected gastric secretion, the urinary catechol amine excretion levels or both. In all groups the compounds have been administered at the time of the start of gastric secretion collection and continued over a 24 hour period. Exception to this was Histamin

which usually was administered prior to operation.

The following groups of animals were prepared:

1) A group of nonfasting animals (42)
2) A group of fasted animals (36)
3) A group of rats with cardia and pylorus ligation without administration of any compound. (45)
4) A group of rats receiving reserpin (57)
5) A group of rats receiving Histamin (43)
6) A group of rats receiving both Histamin and reserpin. (43)
7) A group of rats receiving Dibenzyline (68)
8) A group of rats receiving Dichloroisoproterenol (64)
9) A group of rats receiving Darenthin (48)

Reserpine (Serpasil). The compound used was that supplied through the courtesy of the Canadian Ciba Company, in the strength of 5 mg. per 2 cc. of solvent. The drug was administered to the animals intramuscularly 2 hours after the ligation of cardia and pylorus in the dosage of 0.05 - 0.25 per 100 g. of body weight.

Histamin dihydrochloride. The cristalline powder was used in our experiments supplied to us by the F. Hoffman-La Roche Company of Canada. The substance was dissolved in bidistilled water and a concentration of 20 mg. per c.c. was prepared. Histamin was injected subcutaneously in the dosage of 10 mg. per 100 g. of body weight. The first injection was given 1 hour prior to ligation of the cardia and the pylorus. In some groups

the injection was repeated after 8 and 16 hours.

Histamin and Reserpine. A group of 43 rats were used to evaluate the simultaneous effects of administration of both Histamin and Reserpine. The dosages of both compounds was the same as outlined above for the 24 hour period.

Dibenzyline was obtained from Smith-Kline & French Co. and was supplied as a crystalline powder. The substance was dissolved in minute quantities of ethanol to which the necessary volume of physiologic saline was added in order to achieve a concentration of 2 mg. per c.c. The so prepared solution was to be injected within the next 5 minutes after preparation. The dosage used was 1 mg. per 100 g. of body weight. The administration was intraperitoneally following the operation.

Dichloroisoproterenol was obtained through the courtsey of the Eli Lilley Co. of Indianapolis, Ind., in the form of crystalline material. A concentration of 5 mg. per c.c. was then prepared by dissolving in bidistilled water. The compound was then administered to the rats as a single intraperitoneal injection. The dosage used was 2.5 mg. per 100 g. of body weight 2 hours after the ligation of pylorus and cardia.

Darenthin was supplied by the courtsey of the Burroughs, Wellcome Co. of London as an injectable solution containing 500 mg. in 10 ml. of solvent. A dose of 0.5 - 1.0 mg. per 100 g. body weight was administered subcutaneously 2 hours following the operating procedures.

6. Examination of specimens

In all cases the stomach was examined for the presence of pathological lesions such as hemorrhages or ulcers, following excision of the organ and collection of gastric secretion. No ulcerations were observed in the group with ligated pylorus and cardia, without administration of any drug.

In the groups in which Reserpin was injected a high percentage of hemorrhages, ulcerations of the glandular and forestomach was found, especially when dosages higher than 0.1 mg. per 100 g. of body weight were administered. In the Histamin group there was a high incidence of hemorrhages found in the glandular stomach and of ulcerations in the forestomach especially when large amounts of gastric juice was present. Perforations, mostly localized in the forestomach were found frequently in this group of animals and in some cases caused the death of the animal. Blood-tinged gastric juice was usually present when erosions or ulcerations were found.

In the group in which both compounds Histamin and Reserpine were administered the incidence of hemmorhages and and ulcerations was relatively lower than when both compounds were injected alone. Dibenzyline, D.C.I.P. and Darenthin caused relatively few hemorrhages and ulcerations of the glandular stomach. In the Dibenzyline group there were many cases with numerous deep ulcerations of the forestomach present. The incidence of ulcerations of the forestomach and perforations in this part of the stomach were commonly found with a great amount of gastric secretion.

CHAPTER VII

RESULTS AND DISCUSSION

1. Normal gastric secretory values in rats and comments on the method used for collection of gastric secretion.

The collection of gastric secretion in rats poses several problems. It is obvious that the secretion can be collected only for short periods of time, in the order of 24-48 hours. Preparation of animals with gastric fistula has not met with success, since the rats usually disfigure the fistulous opening by chewing on it. Therefore most of the methods of collection of gastric secretion in rats advise a short period of collection.

Gastric fistula preparation was used previously in this laboratory to collect gastric secretion over a 27 hour period. The preparation consisted of transsection of the stomach at the pyloric end which was then exteriorized and attached by means of a polyethylene tube to a beaker. The following values were obtained: (Webster and Skoryna 1960).

Table I

Table I. Determinations of Gastric Secretory Components in Rats with Gastric Fistula

Rat No.	Volume (cc)	pH	Free HCl (MEQ/L)	NA (MEQ/L)	K (MEQ/L)	CL (MEQ/L)	Pepsin (Units).
1	1.4	3.80	0	84	24.0	141	*
2	0.8	4.25	0	*	*	375	*
3	2.5	3.90	0	60	16.6	65	*
4	2.5	1.20	95	*	*	260	.123
5	2.0	1.45	60	*	*	*	.081
6	5.0	1.40	64	66	9.4	172	.148
7	3.8	4.25	0	78	13.7	135	.143
8	2.0	1.65	50	83	8.2	147	.138
9	4.5	1.50	76	64	9.4	172	.148
10	9.5	1.40	92	60	9.4	155	.167
11	8.0	4.05	0	66	34.1	118	.067
12	14.0	1.50	60	60	17.7	150	.153
13	10.0	1.30	70	63	7.3	147	.148
MEAN	5.08	2.44	43.6	78.4	15.0	169.8	.1316
S.D.	3.90	1.29	36.4	36.0	7.11	75.06	.0854

* Insufficient juice for chemical determination

Shay has used his preparation of pyloric ligation for collection of gastric secretion. The following values were obtained previously in our laboratory using the Royal Victoria Hospital strain of animals (Webster and Skoryna 1960).

Table II

Table II. Determinations of Gastric Secretory Components in Shay Rats

Rat No.	Volume (cc)	pH	Free HCl (MEQ/L)	NA (MEQ/L).	K (MEQ/L)	CL (MEQ/L)	Pepsin (Units)
1	2.5	1.90	45	*	*	160	.157
2	3.5	1.50	85	60	12.5	160	.153
3	7.0	2.60	18	102	6.7	150	.170
4	3.0	1.90	38	83	6.8	160	.128
5	8.0	1.90	48	87	6.6	165	.138
6	7.0	1.90	50	84	6.3	160	.128
7	10.0	1.50	70	65	6.8	160	.143
8	3.5	3.4	0	94	7.7	130	.138
9	5.5	1.9	50	96	9.0	165	.123
10	8.0	6.0	0	107	8.0	155	.128
11	2.0	6.0	*	107	8.0	155	*
MEAN	5.45	2.77	40.4	88.5	7.84	156.36	.1406
S.D.	2.696	1.68	27.68	16.22	1.84	9.77	.015

* Insufficient juice for chemical determination.

There are two disadvantages in using Shay's method for collection of gastric secretion in rats. One is, that the secretion collected is not pure because of admixture of saliva. The second is, that because of the development of the so called Shay ulcers in the forestomach, there is usually some admixture of blood, unless the secretion is collected for a short period of time only, in the order of four (4) hours. This again reduces the amount of secretion collected and abolishes one of the main advantages of this method, which is a relatively large amount of secretion.

The difficulties outlined above were taken into consideration by Skoryna and Webster in devising a method of collection of gastric secretion which consists of ligation of both the cardiac and pyloric ends of the stomach. The secretion obtained from these animals is free from admixture of saliva. Also in none of our groups was an admixture of blood encountered except in rats which developed ulcers and hemorrhages following administration of Reserpine and in some cases following administration of Histamin.

The following values were obtained using the above method of collection of gastric secretion in 45 rats: (mean values)

Volume of secretion	:	2.37
pH	:	1.80
Free HCl	:	40°
Total acidity	:	75°
Chlorides	:	147 m.Eq./Lit.
Sodium	:	82.5
Potassium	:	3.6 m.Eq./Lit.

The values obtained using this method are comparable to values obtained by other methods, except that the volume of secretion collected is smaller. This does not represent a disadvantage, since it is sufficient for carrying out the determinations required.

2. Normal catechol amine levels in rats and comments on the method used for estimation of urinary catechol amine

It is generally accepted, that the levels of catechol amines in the serum and the urinary excretion level vary considerably. U.S. von Euler in commenting the variability of results obtained in human subjects and in animals with measurements of serum catechol amine levels, stated, that the variations are undoubtedly due to a number of factors such as time of collection of the specimen, sex, muscular activity and various other stress factors. Estimation of catechol amines were carried out in the adrenals and autonomic ganglia (extent of depletion); measurements of tissue content such as heart, spleen, liver, kidneys and brain; estimation of urinary excretion levels. It appeared to us that for this

particular series of experiments the estimation of urinary excretion levels was most suitable. The animals did not have to be sacrificed during the period of investigation and gastric secretion studies could be carried out simultaneously by previous ligation of the cardia and the pylorus. It had been attempted at the beginning of this project to estimate the serum levels of catechol amines but this was found to be unsatisfactory for several reasons. The first reason was, that the estimation reflected only the catechol amine level at a particular single point, where the object of the experiment was to estimate the catechol amine "status" of the animal during the whole period of collection of gastric secretion. Secondly the serum levels of catechol amines were found to vary considerably and this undoubtedly reflected the opinion of von Euler as to the number of factors affecting the level of the hormones. The animals had to be sacrificed for the purpose of collection of sufficient amounts of blood and it is well known, that catechol amine depletion occurs prior to death.

In the present studies, only the estimations of Adrenaline and Noradrenaline in the urine were carried out. A number of investigations suggested, that it is more advantageous to estimate also the excretion levels of Dopamine and the V.M.A., a metabolic product of catechol amines. Unfortunately the method of estimation of these compounds was not available at the time of these studies. Adrenaline and

Noradrenaline represent only a small portion of the catechol amines excreted in the urine. Therefore, the possibility of error is increased. As it is evident from the results listed however, we encountered relatively similar levels in a large group of control animals. This might be due to the fact, that it was attempted to adhere strictly to a standardised procedure. The collections were always carried out at the same time. The weight and sex of the animals of an inbred colony were similar. We also attempted to diminish the possibility of error by estimation of catechol amine excretion levels on the basis of the weight of the animals, in addition to taking the urinary output volume into consideration.

The following values were obtained in groups of control animals either fasting or non-fasting for a period of 24 hours.

Table III

Table III. Relationship between Catechol Amines and Body Weight excretion in Control Animals.

Group of Animals	No. of animals	Average B.W.	Adr. Excret. per 100 g. B.W.	N.A. Excret. per 100 g. B.W.
Non-fasted animals	20	165 g.	0.1630 mcrg.	0.0810 mcrg.
Non-fasted animals	15	170 g.	0.0677 mcrg.	0.1042 mcrg.
Non-fasted animals	20	220 g.	0.0750 mcrg.	0.1700 mcrg.
Fasted animals	8	250 g.	0.0480 mcrg.	0.0165 mcrg.
Fasted animals	7	240 g.	0.0410 mcrg.	0.0108 mcrg.
Fasted animals	11	230 g.	0.0800 mcrg.	0.0103 mcrg.
Fasted animals	10	250 g.	0.0640 mcrg.	0.0131 mcrg.

The results obtained in non-fasted animals cannot be taken into consideration due to the fact that food particles were frequently found in the tubes collecting the urine and it is obvious that such particles absorb various amounts of urine and therefore, change the estimated values of catechol amines. Some variation was encountered in fasted animals in which the cardia and pylorus had been ligated, but no drugs administered. However, as it will be stated later - in the discussion of administration of various compounds, significant differences were still noted between this group and those animals in which drugs, which affect catechol amine levels were administered.

3. Effects of Reserpine on gastric secretion and urinary catechol amine levels.

Reserpine is the effective alkaloid of the Indian plant Rauwolfia serpentina which has been used for centuries in India as a popular tranquilizing agent. Reserpine was rediscovered in 1948 and has since been used extensively as a hypotensive and tranquilizer. It is now generally accepted, that the action of Reserpine is at least dual in origin. It has a definite depressive effect on the hypothalamic centers and it acts also peripherally on the sympathetic nervous system.

The study of the effects of Reserpine was of particular importance in our investigations, since the

compound influences both the levels of circulating catechol amines as well as gastric secretion. The effects of Reserpine on the catechol amines studied, are not uniform. Administration of Reserpine depletes the adrenals of their Adrenaline content, which is liberated into the bloodstream and the tissues. Bain (1960) suggested, that the action of Reserpine might be attributable to an interaction between A.T.P. and Reserpine, and that it does not affect the concentration of A.T.P. Burn (1958) has shown, that adrenergic tissues loose their amines under the influence of Reserpine. Farant (1960) has reported, that Reserpine potentiates the response to Adrenaline and Noradrenaline. Bertler et al. (1956) have demonstrated, that administration of Reserpine to rabbits causes disappearance of Noradrenaline from the heart muscle, the rabbit's ear, the spleen and the skin. According to Burn and Rant (1958) the injection of Reserpine reduces the extractable Noradrenaline content of all tissues to a very small amount.

The effect of Reserpine on gastric secretion was studied by several investigators (Berkowitz et al. 1958, Rider et al. 1959, La Barre and Desmarez 1957). La Barre and Desmarez (1957) showed, that in the rat, Reserpine causes ulcerations of the glandular mucose in 66% of animals and multiple hemorrhages in 85%. Gastric secretory volume and acidity were markedly increased in 93% of the animals studied.

The results of our studies of the effect of administration of high doses of Reserpine on gastric secretion and urinary catechol amine levels are summarized in tables IV and V. The volume of gastric secretion was average 10.6 ml., the average pH was 1.5, the free HCl: 40°, total acidity 90°, chlorides 140 m.Eq./Lit., sodium: 95 m.Eq./Lit., Potassium: 1.8 m.Eq./Lit. These findings are in agreement with those of other investigators who have shown considerable increase in gastric secretory acidity following Reserpine administration to rats. They also agree with the findings in human subject kept on Reserpine administration for prolonged periods of time, as reported by Berkowitz, Rider and co-workers. In the four groups of animals in which urinary catechol amine excretion levels were studied after Reserpine administration, the Adrenaline estimations averaged 0.0891 per 100 g. body weight. The mean value for Noradrenaline was 0.1060. This represents a definite increase in urinary catechol amine excretions. Coupland (1949) has reported a very pronounced depletion of Adrenaline from the adrenals following Reserpine administration to rabbits. Our results would indicate, that the urinary excretion of Adrenaline is not as marked, as one would expect to follow after severe depletion of adrenaline stores.

Several pathways for the action of Reserpine on gastric secretion have to be considered. According to Rider (1959) Reserpine influences the gastric secretion by a direct effect on parietal cells. Greenbaum and Wolf suggested, that

Reserpine acts through a Histamine and Serotonin releasing mechanism. According to the author there is also a third possible pathway, namely an increase of blood supply to the stomach through prevention of vasoconstriction, which would occur in depletion of Adrenaline. Presence of Noradrenaline is necessary for vasoconstriction and it is feasable, that lack of it might result in an increased gastric secretory volume. This possibility has to be considered on the basis of the results obtained in our studies. The urinary excretion values for Noradrenaline following Reserpine administration were markedly higher than those in control animals, the average increase being 0.0842. This would indicate a marked depletion of Noradrenaline stores. On the other hand some direct effect of Reserpine on the gastric secretory mechanism can not be excluded in view of the changes in the gastric wall, which were frequently found after Reserpine administration, such as multiple hemorrhages and ulcerations. These would suggest a possible direct effect on the arterio-venous shunting mechanism in the gastric wall, which is affected by Serotonin and Histamin. The suggestion of Rider that a direct stimulating effect on the acid secretory parietal cells is responsible for the changes of the mucosa cells following Reserpine administration, represents probably an oversimplification of the issue. As it will be mentioned later in the discussion of the results of Histamine administration an increase of gastric secretory volume does not necessarily coincide with development of

ulcerations or hemorrhages of the gastric wall, such as were found in the Reserpine treated animals.

Table IV. Mean Values for Gastric Secretion following administration of Drugs.

Compound and Amount	No. of animals	Mean Volume of Secret.	pH.	Free HCl.	Total Acidity	CHl.	Na.	K.
Reserpine 0.05-0.25 mg. per 100 g. B.W.	57	10.5	1.5	45°	90°	140 m.Eq./L.	95 m.Eq./L.	1.8 m.Eq./L.
Histamin 10 mg. per 100 g. B.W.	43	3.9	1.7	40°	70°	135 m.Eq./L.	90 m.Eq./L.	2.5 m.Eq./L.
Histamin and Reserpine 10 mg. per 100 g. B.W.	43	7.3	1.5	55°	65°	130 m.Eq./L.	95 m.Eq./L.	3.5 m.Eq./L.
Dibenzyline 1 mg. per 100 g. B.W.	68	1.5	1.6	60°	90°	140 m.Eq./L.	85 m.Eq./L.	2.9 m.Eq./L.
Dichloroisoproterenol 2.5 mg. per 100 g. B.W.	64	2.8	1.8	40°	75°	40 m.Eq./L.	87 m.Eq./L.	3.2 m.Eq./L.
Darenthin 5-10 mg. per 100 g. B.W.	48	3.5	1.7	40°	70°	136 m.Eq./L.	85 m.Eq./L.	5.2 m.Eq./L.
Group of Control Animals. No Drugs Administered.	45	2.37	1.80	40°	75°	147 m.Eq./L.	82.5 m.Eq./L.	3.6 m.Eq./L.

$) Effect of Histamine on gastric secretion and urinary catechol amine levels.

There are few subjects in gastro enterology which aroused more controversy, than the effects of Histamine on gastric secretion. Popielski (1910) has discovered a substance which he called vasodilatine which he thought to be the active indgredient of peptone extracts responsible for stimulation of gastric secretion. However, the work of Dale & Laidlow (1910-11) has suggested, that the vasodilatin discovered by Popielski has an action identical to Histamine. A long controversy has followed until in 1929 Carnot Koskowski and Libere have decesively demonstrated the stimulating effect of Histamine in man. A tremendous literature follows their original description which is not only impossible to report whithin the space limit of this report, but also it is frequently difficult to establish with certainty the nature of action of Histamine on secretion. The subject is open for further investigation. Although a number of considerable findings regarding the Histamine action has been verified, one of the best recent reviews seems to be that of Code (1956) presented at the Ciba symposium on Histamin, from which the contents below are derived. It appeares to be definitely established, that Histamin stimulates gastrec secretion in all species with few exceptions. The stimulating effect

TABLE V

Catechol Amine Excretion in Urine

(micrograms per 100 grams body weight)

Compound	Group I			Group II			Group III			Group IV		
	No. of rats	A.	N.A.	No. of rats	A.	N.A.	No. of rats	A.	N.A.	No. of rats	A.	N.A.
Non-fasted animals	7	0.1630	0.0801	15	0.0677	0.1042	20	0.0750	0.1700	-	-	-
Fasted animals	7	0.0410	0.0180	8	0.0800	0.0108	11	0.080	0.0103	10	0.0640	0.0131
Reserpine 0.05-0.25 mg./100g.BW.	13	0.0910	0.0860	13	0.0890	0.1260	18	0.0820	0.0980	13	0.0960	0.1150
Histamine 10 mg./100g.	7	0.3680	0.6860	19	0.2630	0.361	8	0.3240	0.3070	9	0.323	0.3780
Histamine & Reserpine	17	0.1770	0.0560	10	0.1580	0.0260	8	0.1390	0.0330	13	0.1330	0.0580
Dibenzyline 1 mg./100g.	19	0.4190	0.6920	20	0.3210	0.4230	16	0.3900	0.7000	13	0.4200	0.7770
DCIP. 2.5 mg%100g.	20	0.2750	0.4590	19	0.2660	0.3370	12	0.3000	0.2470	13	0.3400	0.4830

in rats has been reported by Komarov (1944). In the strain of rats used in the present experiments the stimulating effect in rats has been of Histamin was reported by Jow Webster and Skoryna (1959). MacIntosh and Paton (1953) outlined a series of conditions and compounds which cause the release of Histamin. Antibodies of protein nature, damage to the tissue, toxins, trauma, proteolytic enzymes, curare, morphine and a great number of aromatic and aliphatic chemical comporends. Stomach juice secreted in response to intravenous injection of gastrin shows invariably Histamin activity. Emmelin and Karlssohn (1944). It is possible therefore, that Gastrin is also one of the Histamin releasers. It has been shown by Komarov (1933) that Histamin is present in gastric juice. A definite correlation seems to exist between secretion of parietal cells and Histamin activity of gastric juice (Code 1955). Babkin (1938) has suggested that Histamin is the final chymostimulator, which produces acid gastric juice in response to vagal stimulation.

Therefor, although several important links are missing for complete understanding of Histamin action on gastric secretion, there are still sufficient data available to form a picture of Histamin action. The relationship of this activity and C.A. levels is less clear. Höchfelt (1951) has found that Histamin administration causes a marked release of

Noradrenaline from the adrenals and the adrenergic tissues is less pronounced following Histamin administration. Correlation exists between Histamin and Noradrenaline content in nerves. (V. Enler 1956). A constant relationship has been found in the content of the vagus nerves of the sheep, that of Histamin being 2,4 mcrg/g. and of Noradrenaline 1,0 mcrg/g. Histamin is present in large amounts in postganglionic sympathetic nerve fibres the values being as high as 100 mcrg/100g. No constant relationship was demonstrated between Histamin and its sympathetic nerve supply. After sympathetic nerve section a loss of Histamin in the organ was not observed. (v. Enler and Purkhold 1951). The above authors have concluded, that the Histamin content is not influenced by the presence of sympathetic innervation. The Noradrenaline content of the organ following sympathetic nerve section was almost completely abolished.

The effects of administration of high dosages of Histamin on gastric secretion recorded in the present experiment are summarized in table IV. It is apparent from these figures, that with this dosage range a considerable indrease of gastric volume and some increase in gastric acidity can be elicited. The urinary excretion levels of Adrenaline whithin the 24 hours period average: 0,30190 mcrg/100g. body weight. The excretion levels of Noradrenaline are: 0,4330 mcrg/ 100g. body weight. It seems therefore that, if the

increase in urinary output of C.A. can be considered as an indicator of C.A. depletion, this must occur to a considerable degree following Histamin administration. The fact that values for Noradrenaline were higher than those observed by Höchfelt (1955) following Histamin administration and the values of Adrenaline were lower, can not be explained satisfactorily. It appears that the stimulation of gastric secretion following Histamin administration can and does occur in the presence of depletion of C.A. in adrenergic tissue.

5) Effects of simultaneous administration of Histamin and Reserpine on gastric secretion and urinary catechol amine levels.

Several investigators have reported, that two gastric secretory stimulants, when administered simultaneously, resulted frequently inhibition of gastric secretion. Karwinen & Karwonen (1952) observed inhibition of the stimulating effect of Histamin on gastric secretion collected from isolated gastric pouches in dogs following Insulin administration.

Jow, Webster and Skoryna (1960) have found, that when glucagon was injected in rats 1 hour following Histamin, the volume of gastric juice free Hcl were comparable to the controls, whereas pepsin activity was only slightly elevated. It appeared therfore, that glucagon inhibited Histamin stimulated gastric secretion similarly to Insulin.

In the present experiments it was attemped to study the possibility whether the stimulating effect of Histamin on gastric secretion can be inhibited by Reserpine, a compound which produces both stimulation of gastric secretion and depletion of catecholamine-stores. From table IV and V it can be seen, that the stimulatory effect of Histamin on gastric secretion

was not only not abolished by administration of Reserpine, but some increase in gastric secretory volume, the values obtained being in the middle range between those resulting from Histamin and Reserpine stimulation. The urinary excretion levels of Adrenaline and Noradrenaline following administration of both compounds was higher than that following administration of Reserpine alone and lower than following Histamin alone. It can be concluded therefore, that inspite of presence of considerable depletion of catecholamine-stores, the gastric secretory volume remained increased and that the administration fo C.A. depleting gastric secretory stimulating does not induce inhibition of the stimulating effect on Histamin on gastric secretion. This finding might be in line with the suggestion of Forrest and Code (1954) who found, that the inhibitory effect of Insulin in Histamin induced gastric secretion is not dependent on either vagal or sympathetic nerve supply, but is related to the decreased blood sugar levels. Similarly Jow, Webster and Skoryna (1960) have suggested, that the inhibitory effect of glucagon on Histamin stimulated gastric secretion might be produced by an increased Insulin release by glucagon. The administration for glucagon induces a hypoglycemic period after an initial hyperglycemia in rats, fasting for several hours. Since Reserpine is not known to have such an effect on blood sugar levels, the inhibition of Histamin stimulated gastric secretion should not be expected.

6) Effects of administration of Dibenzyline on gastric secretion and urinary catechol amine levels.

The peripheral adrenergic blocking agents are assumed to act by preventing the postganglionic transmission from reacting with a specific receptor. Site. It number of such agents has been described in the past 10 years. One of the best known is Dibenzyline, which is known to increase the metabolism of Adrenaline. (Shayer, Kennedy and Inscoe (1913). However, Dibenzyline does not interfere with the metabolic spectrum of the C.A. Under normal conditions Epinephrine is attached to certain protective receptors. When Dibenzyline competes with Adrenaline for position or linkage with these receptors a part of Epinephrine loses its protective mechamism and is metabolized more rapidely. Noradrenaline content of the urinary excretion of Adrenaline is not markedly elevated. Schapiro (1958) has found, that administration of Dibenzy line to rats for a period of one week leads to reduction in Norepinephrine content of many organs and an increase in the urinary output of C.A.

Dibenzyline is known to block the excitatory adrenergic receptors. It was therefore of interest to the author to learn, that recently an adrenergic blocking

compound has been described, which specifically blocks the inhibitory adrenergic receptors. This compound, a dichloro analogue of Isoproterenol has described by Powell & Slater (1958). Thus it was thought, that by studies of gastric secretion in both groups it might be possible to decide whether the effect of Adrenergic discharge on gastric secretory activity are mediated through the excitatory or inhibitory adrenergic receptors.

It is apparent from table IV that administration of Dibenzyline resulted in a marked decrease of gastric secretory volume, although the free Hcl was significantly increased. As evident from table V the uninary excretion values of both Adrenalin Noradrenalin were considerably increased. Following the administration of dichloroisoproterenol the gastric secretory volume was unsignificantly increased, the free Hcl and total acidity remained whithin normal limits. The effects of D.C.I.P. on urinary C.A. was not as prominent as following Dibenzyline administration, although still significant.

It can be concluded therfore that adrenergic blocking componds, which act on the excitory adrenergic receptors have the tendency to inhibit gastric secretion, while those acting on the inhibitory adrenergic reseptors do not affect gastric secretory activity of the stomach.

7) Effects of Darenthin on gastric secretion and urinary catechol amine levels.

Recently a hypotensive agent has been developed with selectively which blocks the adrenergic nevous system without antagonizing the effect of released or injected Adrenaline or Noradrenaline. The compound which is chemically known as Bretylium tosiliate has been marketed under the name of Darenthin and has been developed in the wellcome reseach laboratories. To our knowledge the effect on gastric secretion of this new drug has not been studied yet. The original publication of Boura etal (1959) mentions that no depression of the parasympathetic nervoussystem has been observed following Darenthin administration to rats. In our experiments administration of Bretylium tosilate to rats resulted in an increase of gastric secretory volume, although the free and total Hcl remained whithin the normal range. (Table V) Interesting observations have been made, namely, that the K. content of gastric secretion was markedly elevated. As a matter of fact this was the only group in which a significant increase of potasium levels of the gastric juice was observed.

Unfortunately the results of the estimations of C.A. levels following Darenthin administration were not available at the time of submission of this report.

According to Boura et al (1959) the C.A. content of the adrenals of rats is not shanged following Darenthin administration. Simirlarly the content of the synaptic ganglia was found not to deffer from that of normal animals. There seems to be preferential accumulation of the compound in the adrenergic nerves, which inhibit their function. Boura suggested, that the effects of Darenthin can be explained as producing a selective block of adrenergic nerves. Since the functions of these nerves induce vasoconstriction and the hypotensive action of Darenthin is probably based on vasodilatation, it is possible, that the increase in gastric secretion observed in our studies following Darenthin administration are due to vasodilatation of the vascular supply to the stomac wall.

CHAPTER VIII

SUMMARY AND CONCLUSIONS.

1) The chemistry and data concerning formation storage and release of C. A. have been reviewed.

2) The metabolism of C. A. has been discussed in detail including the general metabolism aspects, the metabolism in patients with pheocheromocytoma and the role of enzymes.

3) The functions of C. A. have been discussed according to the present concepts of their actions. The functions have been divided according to the classification of Koelle as excitatory functions, inhibitory functions, effects on metabolism of fats, proteins and the calorigenic effect, effect on the central nervous system. Comments have also been made on the effects of C. A. on various systems, such as the gastro-intestinal tract, the respiratory system and the cardio-vascular effects.

4) In the present experiment the gastric secretion in rats has been studied following administration of compounds which are known to affect both, the gastric secretion and the catechol amines.

The method used for collection fo gastric secretion was that of Skoryna and Webster consisting of ligation of cardia and pylorus in rats fasted for 24 hours and analysis of the secretion accumulated in the stomach during 24 hours. Gastric secretory volume, p.H., free and total acidity, chlorides, potassium and sodium were studied.

The method used for estimation of urinary excretion of C. A. was a modification of the original method of U. S. von Euler, suggested by Dr. R. Hobkirk of the Montreal General Hospital. The method consists of fluorimetric estimation of catechol amines following preparation of the urine, absorption on alumina and onidation by iodine. The readings were carried out on the Coleman electronic photospectrofluorometer.

5) Reserpine administration in doses of 0.05 - 0.25 mg/ 100 g body weight resulted in marked stimulation of gastric secretion. The urinary excretion levels of catechol amines were increased. It was concluded, that the effect of Reserpine on gastric secretion is a direct one, possibly affecting the arterio-venous shunting mechanism in the gastric wall. The above conclusion was reached in view of the numerous ulcerations and hemorrhagic changes in the stomach of the rat following Reserpine administration, in addition to the stimulating effect on gastric secretory mechanisms.

It appears that the stimulation of gastric secretion can occur under the conditions of depletion of catechol amine stores, as indicated by the increased urinary output of the hormones.

6) The administration of Histamine in the dosage of 10 mg/100 g body weight resulted in significant increase of gastric secretion, which was less pronounced than that in Reserpine administration. The urinary excretion levels of Adrenaline and Noradrenaline were extremely elevated. It was concluded that, although stimulation of gastric secretion is possible in the presence of a depletion of catechol amines, the stimulating effect is inversely proportional to the extent of the depletion.

7) Administration of both compounds, Histamine and Reserpine over a 24 hours period in the dosage stated above, resulted in stimulation of gastric secretion, which was more pronounced than in Histamine administration alone, but less pronounced than following Reserpine administration. The urinary excretion levels of Adrenaline and Noradrenaline were higher than those following Reserpine injections and lower than in Histamine-treated animals. It is concluded, that the inhibition of gastric secretion which was observed to follow the administration of two secretory stimulants, such as Histamine and Insulin

or Histamine and Glucagon, is not related to the catechol amine levels and possibly be connected with the blood sugar levels of the animal.

8) The administration of high dosages of Dibenzyline (1 mg/100 g body weight) resulted in a marked inhibition of gastric secretory values, while the administration of Dc I P (2.5 mg/100 g body weight) had no effect on gastric secretion. The urinary excretion levels of the neurohormones were increased following administration of each of the compounds. Since Dibenzyline is known to block the excitatory adrenergic receptors and D C I P the inhibitory receptors, it is concluded, that the adrenergic blocking compounds which act on the exciatory adrenergic receptors have the tendency to inhibit gastric secretion, probably by causing vasoconstriction.

9) Administration of Bretylium tosilate (Darenthin) in the dosage of 0.5 - 1.0 mg/100g body weight resulted in increase of gastric secretion, which probably may be caused by dilatation of the vascular tree supplying the stomach wall, due to the preferential accumulation of the compound in the adrenergic nerves.

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