Metabolism and Storage of Biogenic Amines* **

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One hundred years ago, on September 29th, 1856, VULPIAN[2] communicated to the Paris Academie des Sciences his observations on the colour reactions of the adrenal medulla. VULPIAN describes the characteristic reactions with iodine, with ferric chloride and with alkali. He concludes: «Il existe donc une matière spéciale, inconnue jusqu'ici, douée de propriétés chimiques remarquables, qui se trouve exclusivement dans la substance médullaire des capsules surrénales, et qui, par conséquent, constitue le *signe particulier* de ces organes.

Mais cette matière, qui se montre ainsi dans la substance médullaire, est-elle destinée à s'y détruire sur place, ou bien pénètre-t-elle dans le sang, pour être entraînée dans le torrent circulatoire? Je suis très-disposé à admettre la seconde supposition...» The reason why he believes this second alternative to be true, is because the characteristic colour reaction can also be seen in the suprarenal venous blood. «Ainsi serait prouvée, pour la première fois et d'une façon décisive, l'hypothèse qui regarde les capsules surrénales comme des glandes dites sanguines, c'est-à-dire versant directement dans le sang leur produit de sécrétion.

Quelle est l'importance de cette sécrétion? J'avoue que je n'ai encore pu me former aucune idée sur les usages possibles de cette matière. Je ne hasarderai par conséquent aucune hypothèse.»

In retrospect, it seems remarkable that VULPIAN'S prophetic conclusions were not immediately followed up. Forty years elapsed before the remarkable biological activitity of the adrenal medullary hormone was discovered. On the other hand, VULPIAN'S work is not forgotten, and the colour reaction with iodine is the basis of a useful modern method of determination of the catechol amines (v. EULER and HAMBERG[3]). The product of the oxidation of adrenaline with iodate, the iodoadrenochrome of adrenaline, was obtained in crystalline form in 1937[4]. The reaction product of the oxydation by potassium bichromate, to which the chromaffine cell owes its name, has not yet been isolated. The chromaffine reaction was described by HENLE[5] nine years after VULPIAN'S discovery.

Adrenaline is a typical representative of those compounds to which GUGGENHEIM[6] has given the name 'biogenic amines'. This group includes substances which have high biological activity, and it is with these substances that this review is concerned. Three different groups of compounds can be distinguished:

(1) The catechol amines: adrenaline, noradrenaline and dopamine;

(2) histamine; and

(3) 5-hydroxytryptamine (HT) and related compounds.

These three groups of amines have much in common. They have high activity upon both muscles (plain and cardiac) and glands. They are all derived from the amino acids present in proteins. They are all stored in specific storage sites in cell granules, and they are all inactivated by enzymes in the tissues.

We have to remember that the biological significance of these substances is still imperfectly understood. Most is known about adrenaline and noradrenaline, important as hormones and the latter also as mediator at adrenergic nerve endings. However, the function of the more recently discovered 'brain sympathin'[7], a mixture of adrenaline and noradrenaline, is still unknown. It is of interest that brain sympathin and brain HT[8] are present in the same sites, i. e., in the hypothalamus and brain stem. It is generally agreed that HT is a biogenic amine of great importance, but its normal function is still unknown. Histamine, although known for almost 50 years, is also still without a definitely assigned physiological role; as far as is known at present, its great importance lies in its 'nuisance value', the symptoms which it causes when it is released from cells into the tissue fluids under pathological conditions (see [9]).

In recent years, biochemical methods have added much to our knowledge of these compounds. It is these new findings and the new questions that they pose which will be discussed in the following.

A. *Formation of Amines in the Animal Organism*

The biogenic amines are derived from the dietary amino acids. The amines owe their origin to the pres-

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[2] D. VULPIAN, C. r. Acad. Sci., Paris *43*, 663 (1856).

[3] U. S. v. EULER and U. HAMBERG, Acta physiol. scand. *19*, 74 (1949).

[4] D. RICHTER and H. BLASCHKO, J. chem. Soc. *1937*, 601.

[5] J. HENLE, Z. rationelle Med. 3. Reihe, *24*, 143 (1865).

[6] M. GUGGENHEIM, *Die biogenen Amine* (Karger, Basel, 4. Aufl. 1951).

[7] M. VOGT, J. Physiol. *123*, 451 (1954).

[8] A. H. AMIN, T. B. B. CRAWFORD, and J. H. GADDUM, J. Physiol. *126*, 596 (1951).

[9] *Ciba Foundation Symposium on Histamine* (Churchill, London 1956).

ence of specific enzymes, the amino-acid decarboxylases which catalyse reactions of the general type:

$$\underset{\text{amino acid}}{R\cdot CH(NH_2)\cdot COOH} \longrightarrow \underset{\text{amine}}{R\cdot CH_2\cdot NH_2} + CO_2$$

Decarboxylation is the reaction in which biological activity arises; the amino acids are without the characteristic biological activity. It seems that the zwitter ions must first be converted to the base, before biological acitivity appears. However, decarboxylation is not the only step in the biosynthesis of the biogenic amines. The complexity of the biosynthetic pathway differs for each group of amines; it depends upon the number of differences between the starting material and the finished product. Formation is a one-step reaction for histamine; here the histidine decarboxylase is the only catalyst involved. The histidine decarboxylase, like all other amino-acid decarboxylases, contains pyridoxal-5-phosphate as its prosthetic group.

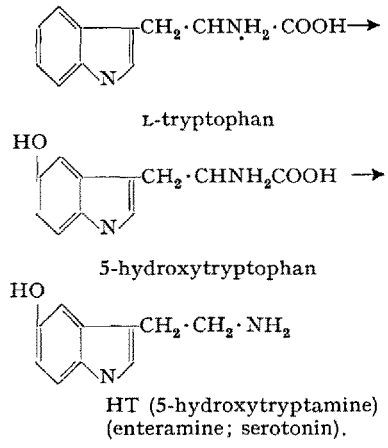
Histidine decarboxylase was discovered in bacteria, but it also occurs in mammalian tissues (WERLE[10]; WATON[11]). The histidine decarboxylase activity of the tissues is low, and for this reason enzymic activity in tissue extracts is often difficult to demonstrate. This has led to the suggestion that in some species, including man, histamine is not formed, but taken up from the intestinal lumen, where it arises as a result of bacterial activity. However, it must be kept in mind that pyridoxal phosphate enzymes tend to dissociate in tissue extracts:

$$\text{decarboxylase} \rightleftharpoons \text{codecarboxylase} + \text{apodecarboxylase}.$$

Such a dissociation of the enzyme may easily occur in extracts when the codecarboxylase concentration is lowered by dilution. It is known that such extracts often have higher activity when the codecarboxylase, pyridoxal-5-phosphate is added *in vitro*. Experiments with isotopically labelled histamine and histidine make it very likely that the principal site of histamine formation is in the cell that stores histamine.

The low rate of histamine synthesis becomes apparent when animals are depleted of histamine. FELDBERG and TALESNIK[12] have depleted animals of skin histamine by the use of the histamine releaser, compound 48/80; it is remarkable how long it takes until the stores of histamine are replenished.

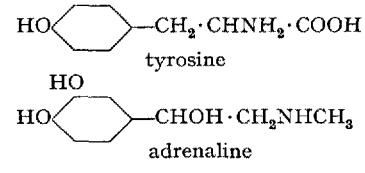
The biosynthesis of HT is somewhat more complex; this is explained by the fact that the parent compound, tryptophan, differs from the amine in two points: (a) in the absence of the carboxyl group, and (b) in the presence of the hydroxyl group in position 5 on the indole ring. Thus, two pathways are theoretically possible, and the work of UDENFRIEND, CLARK and TITUS[13] has shown that in the biosynthesis decarboxylation follows upon the introduction of the hydroxyl group:



HT (5-hydroxytryptamine) (enteramine; serotonin).

Little is known about the enzyme that oxidizes tryptophan to 5-hydroxytryptophan. More is known about the specific 5-hydroxytryptophan decarboxylase; it is a pyridoxal enzyme; it occurs not only in the kidneys[14], but also in large amounts in the intestine and in the nervous system[15]. In the latter, most is found in the hypothalamus and, interestingly, also in sympathetic ganglia. The localization of the enzyme in the hypothalamus is not surprising; like brain sympathin, brain HT is particularly concentrated in the hypothalamus (and the brain stem), but the sympathetic ganglia are not found to be particularly rich in HT. The enzyme has been shown to occur in large amounts in a metastasis of an enterochromaffine tumour[16].

Although the chemical composition of the catechol amines has been established for a long time, the elucidation of their biosynthesis has been slow. The reason for this is easily understood; adrenaline is derived from tyrosine or phenylalanine; direct evidence of this has been obtained by the use of radioactive amino-acid precursors[17].

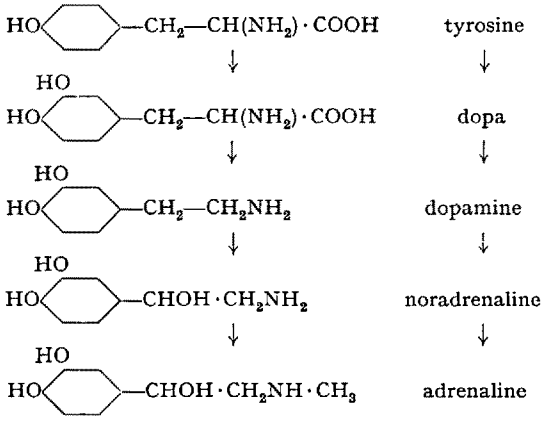


Since adrenaline differs from tyrosine in four different points, many different pathways of biosynthesis are theoretically possible. In 1938, HOLTZ, HEISE and LÜDTKE[18] discovered in the mammalian kidney the enzyme dopa decarboxylase: this enzyme catalyzes the formation of dopamine from L-dopa; this led to the suggestion that this reaction was one of the intermediate steps in the biosynthesis of adrenaline and of

[10] E. WERLE and H. HERRMANN, Biochem. Z. *291*, 105 (1937).
[11] N. G. WATON, Brit. J. Pharmacol. *11*, 119 (1956).
[12] W. FELDBERG and J. TALESNIK, J. Physiol. *120*, 550 (1953).
[13] S. UDENFRIEND, C. T. CLARK, and E. TITUS, J. Amer. chem. Ass. *75*, 501 (1953).
[14] C. T. CLARK, H. WEISSBACH, and S. UDENFRIEND, J. biol. Chem. *210*, 139 (1954).
[15] J. H. GADDUM and N. J. GIARMAN, Brit. J. Pharmacol. *11*, 88 (1956).
[16] H. LANGEMANN and J. KÄGI, Klin. Wschr. *34*, 237 (1956).
[17] S. GURIN and A. M. DELLUVA, J. biol. Chem. *170*, 545 (1947). – S. UDENFRIEND and A. J. B. WYNGARDEN, Biochim. biophys. Acta *20*, 48 (1956).
[18] P. HOLTZ, R. HEISE, and K. LÜDTKE, Arch. exp. Path. Pharmak. *191*, 87 (1937).

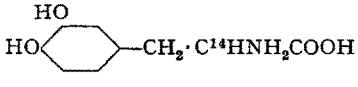
sympathin. The discovery of the role of noradrenaline as the adrenergic mediator (v. EULER[19]) gave support to the idea that a primary amine occurred as the precursor of adrenaline.

Experimental support for the scheme given below was first obtained by LANGEMANN[20], who showed that the chromaffine tissue is extremelly rich in dopa decarboxylase.



This observation, which has since been confirmed and extended to many other vertebrate species, shows that the presence of this amine-forming enzyme is a characteristic of the phaeochrome tissue. It is now known that the dopa decarboxylase is also present in adrenergic nerves[21]. This is further support for the suggestion that the enzyme is concerned with the elaboration of sympathin in the organism.

These findings have led to a search for an enzyme which would convert dopamine to noradrenaline. Two years ago, it was shown that dopa, labelled in the α position with carbon 14, when incubated with bovine



medullary homogenates, gave rise not only to radioactive dopamine, but also to small amounts of radioactive noradrenaline[22]. This observation was followed up by HAGEN[23], who isolated the radioactive dopamine and then incubated it with a homogenate from chick suprarenal gland; in this way the conversion of dopamine to noradrenaline could be directly demonstrated. Observations have since been reported which indicate that the same reaction occurs in the adrenal medullary tissue of the ox[24], the cat [25] and the rat[26]. It therefore seems that this mode of formation of the pressor amines is widely distributed, possibly common to all vertebrates. We can therefore say that today, 100 years after the first demonstration of the medullary hormone, its mode of synthesis can be considered as established in broad outlines, although many interesting details, especially the mechanism of the methylation reaction[27] remain to be cleared up.

These recent findings raise the question: what is the functional significance of dopamine? Its presence in human urine has been known for some time[28], and its occurrence in the adrenal medulla has also been demonstrated[29,30]. In chromaffine tissue, only very small quantities of dopamine occur; this suggests that in this tissue, like a true metabolic intermediate, it is not stored. SCHÜMANN[30] has recently shown that in adrenergic nerves this appears to be different; here the amounts of dopamine found are comparable with those of noradrenaline present. This suggests the possibility that dopamine has some regulating functions of its own which are not yet known. In this connexion, it seems worth while to remember that in many species the kidneys are very rich in dopa decarboxylase, but no renal function of dopamine has hitherto been demonstrated.

B. *Biological Inactivation of Biogenic Amines*

It is not proposed here to discuss the different modes of biological inactivation in detail. The amines owe their characteristic biological properties to the presence of the basic nitrogen, and it is therefore not surprising to find that inactivation is often brought about by removal of the amino group. This removal is catalysed by enzymes known as amine oxidases. Oxidative deamination by amine oxidases leads to the formation of an aldehyde, but the aldehyde is usually further oxidized to the corresponding carboxylic acid. In this way, the excretion of 5-hydroxyindoleacetic acid has been used as a measure of the turnover of HT in the body. Similarly, imidazoleacetic acid has been demonstrated as an important end product of metabolism of histamine. However, the possibility that other pathways of inactivation exist, should be borne in mind.

It is certain that oxidative deamination by amine oxidases is not the only way in which amines are destroyed. Only for HT has no other pathway of inactivation so far been described. For histamine, alternative pathways of inactivation have been discussed in detail at the recent CIBA Symposium on Histamine (see[9]).

It is the fate of the catechol amines in the animal organism which is still very imperfectly known. Adrenaline and noradrenaline are both substrates of amine oxidase *in vitro*, and experience gained by the use of isotopically labelled amines makes it likely that some

[19] U. S. v. EULER, Ergebn. Physiol. *46*, 261 (1950).
[20] H. LANGEMANN, Brit. J. Pharmacol. *6*, 318 (1951).
[21] P. HOLTZ and E. WESTERMANN, Arch. exp. Path. Pharmak. *227*, 538 (1956).
[22] D. J. DEMIS, H. BLASCHKO, and A. D. WELCH, J. Pharmac. *117*, 208 (1956).
[23] P. HAGEN, J. Pharmac. *116*, 26 (1956).
[24] R. NERI, M. HAYANO, D. STONE, R. I. DORFMANN, and F. ELMADJAN, Arch. Biochem. *60*, 297 (1956).
[25] N. KIRSHNER and McC. GOODALL, Fed. Proc. *16*, 110 (1956).
[26] L. C. LEEPER and S. UDENFRIEND, Fed. Proc. *15*, 298 (1956).
[27] W. G. VERLY, Arch. int. Physiol. Biochem. *64*, 309 (1956).
[28] U. S. v. EULER, U. HAMBERG, and S. HELLNER, Biochem. J. *49*, 655 (1951).
[29] McC. GOODALL, Acta physiol. scand. *24*, suppl. 85 (1951). – D. M. SHEPHERD and G. B. WEST, J. Physiol. *120*, 15 (1953).
[30] H. J. SCHÜMANN, Arch. exp. Path. Pharmak. *227*, 566 (1956).

at least of the adrenaline and noradrenaline is metabolized by amine oxidase *in vivo*[31]. However, this oxidation is a slow process, and it appears likely that other mechanisms of inactivation of these two amines will be found to play an important part. Here is a gap in our knowledge which remains to be filled.

The third catechol amine, dopamine, is readily oxidized by amine oxidase, and there is no reason to doubt that this enzyme is active as a catalyst of biological inactivation of dopamine in the living organism.

C. *Storage of Biogenic Amines*

The development of methods for the study of isolated cell organelles *in vitro* has in recent years greatly contributed to our knowledge of the storage of biogenic amines in the cell.

It has been found that in the cell amines are stored in cytoplasmic granules. Granules of this kind have been known to the histologists for a long time, but it has now been found possible to isolate the granules and obtain them in suspensions. A method which has been found useful for isolation of these granules is the differential centrifugation of tissue homogenates in sucrose solution; isotonic or hypertonic solutions of sucrose have been used. In this way it has been demonstrated that histamine[32], adrenaline and noradrenaline[33] and HT[34] are stored in granules.

Each amine is probably stored in more than one type of cell, but it appears that for each of the three groups of amines one specific storage site is especially important. For histamine, this cell is the mast cell, which stores in its granules both histamine and heparin[35]. The catechol amines are stored in the chromaffine cells and in the related adrenergic nerve cells. ERSPAMER[36] and others see in the enterochromaffine (or argentaffin) cells of the gastro-intestinal tract the specific storage cell for HT, but the suggestion has been made by JACOBSON[37] that the storage site is in a different type of cell, known as argyrophil (see also LILLIE[38]).

The granules which store the catechol amines and those which store histamine have certain characteristics in common:

(a) they contain the amines in a form in which they are not immediately available to exert their full biological action[39]; however, upon treatment with distilled water the amines are released; they are then able to exert their full effect. These observations are in agreement with the idea that the granules are provided with membranes that have properties similar to those of cell membranes.

(b) The granules contain specific anions which appear to be important in the binding of the amines. In the mast cell granule, this anion is heparin, in the chromaffine cell it is adenosinetriphosphate (ATP). The high concentration of ATP in the bovine adrenal medulla was discovered by HÖGBERG, HILLARP and NILSON[40], and it has since been shown that the distribution of amines and ATP over the different granular fractions obtained from the chromaffine cell by differential centrifugation is very similar[41]. The concentration of ATP in the amine-carrying granules of the suprarenal medulla is extremely high; 5 to 10% of the dry weight of these granules is ATP. This is a concentration of ATP of an order of magnitude different from that hitherto found in other cells. It is of interest that the blood platelets have recently been found to be rich in ATP[42]. This is interesting as the platelets are known to act as carriers of amines.

The analysis of the chromaffine granules by differential centrifugation has recently been supplemented by a study of the chromaffine cell with the electron microscope[43]. This study has shown that the chromaffine granules are clearly distinct from the mitochondria; they are of smaller size than the latter. Valuable evidence on the existence of a granular membrane has also been obtained.

One hundred years ago, VULPIAN described the 'granulations moléculaires' of the adrenal medullary cell. Recent work has shown that these structures are important in the storage, and possibly also in the formation, of the medullary amines.

Zusammenfassung

Die Entdeckung der Farbreaktionen der Nebennierenmarksubstanz durch VULPIAN vor 100 Jahren gibt den Anlass zu diesem Überblick über den gegenwärtigen Stand unseres Wissens vom Stoffwechsel der pharmakologisch wirksamen biogenen Amine. Die Bildung des Histamins, des 5-Oxytryptamins und der Katecholamine (Noradrenalin und Adrenalin) und der Anteil der Aminoxydasen an der biologischen Inaktivierung der Amine wird besprochen. Zum Schluss werden die Ergebnisse der Granulaforschung in den letzten Jahren erwähnt und die Bedeutung dieser cytoplasmatischen Strukturen für die intracelluläre Speicherung der biogenen Amine sowie einige Eigenschaften der Granula diskutiert.

[31] R. W. SCHAYER and R. L. SMILEY, J. biol. Chem. *202*, 425 (1953). – R. W. SCHAYER, R. L. SMILEY, K. J. DAVIS, and Y. KÓBAYASHI, Amer. J. Physiol. *182*, 285 (1955).

[32] J. H. COPENHAVER, M. E. NAGLER, and A. GOTH, Fed. Proc. *12*, 314 (1953). – P. HAGEN, Brit. J. Pharmac. *9*, 100 (1954).

[33] H. BLASCHKO and A. D. WELCH, Arch. exp. Path. Pharmak. *219*, 17 (1953). – N. A. HILLARP, S. LAGERSTEDT, and B. NILSON, Acta physiol. scand. *29*, 251 (1953).

[34] H. BLASCHKO, J. M. HIMMS, L. MARTINI, and J. M. WALKER, Unpublished observation. – N. J. GIARMAN, Unpublished observation.

[35] J. F. RILEY, Pharmac. Rev. *7*, 267 (1955).

[36] V. ERSPAMER, *Il sistema cellulare enterocromaffine e l'enteramina*, Rendiconti Scientifici Farmitalia, Vol. 1 (1954).

[37] W. JACOBSON, *Ciba Foundation Symposium on Chemistry and Biology of Pteridines* (Churchill, London 1954), p. 314.

[38] R. D. LILLIE, J. Histochem. and Cytochem. *4*, 120 (1956).

[39] H. BLASCHKO, P. HAGEN, and A. D. WELCH, J. Physiol. *129*, 27 (1955).

[40] B. HÖGBERG, N.-A. HILLARP, and B. NILSON, *3me Congrès International de Biochimie*, Résumé des communic. (1955), p. 72.

[41] H. BLASCHKO, G. V. R. BORN, A. D'IORIO, and N. R. EADE, Biochem. J. *62*, 18 P (1956); J. Physiol. *132*, 44 P (1956). – B. FALCK, N.-A. HILLARP, and B. HÖGBERG, Acta physiol. scand. *36*, 360 (1956).

[42] G. V. R. BORN, Biochem. J. *62*, 33 P. (1956).

[43] J. D. LEVER, Endocrin. *57*, 621 (1955). – F. S. SJÖSTRAND and R. WETZSTEIN, Exper. *12*, 196 (1956).