Formation of Norepinephrine from Tyrosine in Isolated Rabbit Heart

The biogenesis of norepinephrine involves the oxidation of phenylalanine to tyrosine, the catalyzed conversion of tyrosine to 3,4-dihydroxyphenylalanine, the decarboxylation of 3,4-dihydroxyphenylalanine to dopamine (3,4-dihydroxyphenylethylamine) by DOPA decarboxylase, and the β-hydroxylation of dopamine to norephinephrine by dopamine β-oxidase. Virtually nothing is known about the formation of dopa from tyrosine in mammalian enzyme systems, except that this reaction occurs in medulla and that it is not catalyzed by tyrosinase[1]. It remains, therefore, to be elucidated whether organs innervated by sympathetic nerves such as the heart are able to perform all the enzymatic reaction steps from tyrosine leading to norephinephrine.

The rabbit heart was perfused with dopamine-C^{14}, tyramine-C^{14}, and tyrosine-C^{14} and the formation of catechols and β-hydroxylated compounds were investigated.

Rabbits were pretreated with 100 mg/kg iproniazid and killed 16 h later. The thorax was immediately opened and the heart removed and perfused by the Longendorff technique with oxygenated (95% O_2, 5% CO_2) Krebs-Ringer bicarbonate solution at 38°C. A stopcock on the cannula allowed continuous infusion of radioactive compounds into the perfusing fluid. The outflow from the hearts was collected during the perfusion time. The radioactive compounds in the heart and perfusate were isolated and analyzed by a method which involves extraction into organic solvents, ion exchange, alumina and paper chromatography as well as acetylation procedures[2].

After perfusion with dopamine-C^{14}, 1–3% was converted to norepinephrine which was accumulated in the heart tissue. In the perfusate, unchanged dopamine-C^{14} and two *O*-methylated metabolites, 3-methoxy dopamine and 3-methoxy norephinephrine were found. No norepinephrine was detected in the perfusate.

Perfusion with tyramine-C^{14} yielded norsynephrin as a major radioactive product in the heart tissue (Table). Only negligible amounts of unchanged tyramine-C^{14} were found. The perfusate contained tyramine-C^{14} and other metabolites which have not been identified at the present time. Neither dopamine-C^{14} nor norepinephrine-C^{14} have been detected after perfusion with tyramine-C^{14}. When tyrosine-C^{14} was perfused, norepinephrine-C^{14} as the main catechol, and dopamine-C^{14} as the minor, were isolated from the heart. In the perfusate a catechol was isolated with chromatographic characteristics of dopa-C^{14}. Whether tyramine or norsynephrin is formed after perfusion with tyrosine is now under investigation.

The present study shows that the heart is capable of performing all the enzymatic reactions necessary for the biosynthesis of norepinephrine. The finding that tyrosine is hydroxylated to dopa while tyramine is not converted to dopamine in the isolated heart shows the specificity of this reaction. Of considerable interest now is to investigate whether other phenolic amino acids are also converted by this system to the corresponding catechols. Attempts are now being made to isolate the enzyme from the heart in order to study the co-factor requirements and the substrate specificity.

Previously it was shown that the enzyme which converts dopamine to norepinephrine is not specific and that tyramine is also a substrate of the same enzyme[3]. It may therefore be assumed that tyramine was converted in the isolated heart to norsynephrin by the same enzyme which formed norepinephrine from dopamine.

The formation of norepinephrine from tyrosine and norsynephrin from tyramine

Perfusion time in min	Perfused precursor			Product[a]	
	Compound	Weight in mg	c.p.m. $\times 10^6$	Compound	c.p.m. $\times 10^3$[b] per 10 g tissue
45[c]	Tyramine	0.04	1.6	Norsynephrin	43.00
120	Tyrosine	0.85	3,5	Norepinephrine	9.6

[a] The radiochemical purity of the products was established by paper chromatography in two different solvent systems of the free compounds and acetylated derivatives.
[b] Figures represent averages of 3 experiments in each series.
[c] The radioactive compound was perfused for 15 min and the perfusion was continued for the next 30 min.

Zusammenfassung. Die Bildung des Noradrenalins aus Tyrosin konnte im isolierten Kaninchenherzen nachgewiesen werden. Die Reaktion scheint für Tyrosin spezifisch zu sein. Aus Tyramin wurde kein Noradrenalin gebildet. Ebenfalls wurde die Umwandlung von Tyramin in Norsynephrin und von Dopamin in Noradrenalin nachgewiesen.

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PRO EXPERIMENTIS

A Method of Measuring the Intensity of Electron Spin Resonance Absorption

In this brief note a new quantitative approach to the study of electron spin resonance (ESR) absorption is described. In its essence, it is a method of *comparing* the intensity of ESR signals. Thus, it makes possible the *relative* measurements of the concentration of paramagnetic centres, but not the *absolute* determinations.

The method has been elaborated in the course of biophysical investigations[1] and is adapted to the range of concentrations in which free radicals are shown to be present in biological materials (i.e. 10^{-6}–10^{-8} mole/g of dry weight[2]). It may, however, be applied as well to the measurements of other paramagnetic species in a wider range of concentrations, provided that the investigated substance is isotropic, powdered, or dissolved.

The quantitative comparison of ESR signals becomes a somewhat involved problem, if the samples under examination have different dielectric properties, since in this case they produce different dielectric losses, and affect the Q-value of the resonant cavity unequally. This is a serious difficulty, because the intensity of ESR signals depends upon Q^2 value. As a result, the above-mentioned difficulty may be the source of considerable errors which hitherto could only be avoided by applying either the extrapolation method[3] or special types of resonant cavities[4,5].

Another solution of the problem is presented in this communication. The essential feature of the method described here is that both standard and sample can be present simultaneously in the cavity during the measurement, as in the case of the double-cavity technique. As opposed, however, to the latter method, no modifications of the cavity or of the modulating system are necessary. One pair of modulating coils and a simple cylindrical cavity of H_{011} mode, as commonly used, are quite satisfactory. The only requirement is the possibility of changing the orientation of the magnetic field in the plane perpendicular to the cavity axis.

The principle of the method is based on the fact that the probability of transitions induced by magnetic dipole coupling varies with the angle between the lines of force of interacting magnetic fields, i.e. the d.c. magnetic field and the magnetic component of the microwave field (Figure 1). Hence, by changing this angle between 90° and 0°, a gradual drop of ESR absorption intensity must be obtained, ranging from a certain maximum value, at the perpendicular position of the lines of force (Figure 2b) to the complete disappearance of the signal, at the parallel or 'zero-position' (Figure 2a).

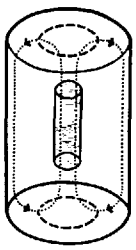

Fig. 1. Magnetic component of the microwave field in a cylindrical resonant cavity of H_{011} mode (dotted lines). Positions in which standard can be placed marked by the broken lines. The sample (shaded) is fixed, as usually, in the axial part of the cavity.

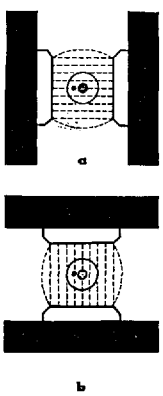

Fig. 2. Scheme illustrating the principle of the 'magnet-rotation method' (top view). a, parallel or 'zero-position', b, perpendicular or 'cross-position' of the magnetic fields. The positions of standard and sample in the resonant cavity are marked.

This can easily be demonstrated if the paramagnetic substance is placed at some distance from the axis of the resonant cavity, preferably in the middle of its radius (e.g. at the bottom or in the top of the cavity). Rotating the magnet in the plane vertical to the cavity axis, one can 'cancel out' or increase the ESR absorption occurring in the substance located in such an 'unusual' position.

Taking advantage of the above relations, it is possible to fix a permanent standard in the resonant cavity placing it eccentrically, as previously described (Figure 1), while the samples are put in the 'normal' place, i.e. in the central part of the cavity. If the material to be examined is isotropic, powdered or dissolved, the absorption intensity remains constant in spite of magnet rotation. This offers the possibility of comparing the intensity of ESR signals from a standard and a sample simultaneously present in the cavity, since the rotation of the magnet influences only the absorption brought about by the standard.

Thus, each measurement consists of two successive scannings of the magnetic field: one at 'zero-position' (Figure 2a), and the other at 'cross-position' of the magnet (Figure 2b). In the first run, the signal from the standard is eliminated, the spectrum of the sample being recorded exclusively. In the second scanning, both standard and sample absorb the microwaves, giving rise to a resultant absorption curve, the intensity of which is determined by two overlapping signals. If we denote the intensity of the first ESR signal by 'I_0', and the intensity of the next signal by 'I_{90}', we may write: $I_{90} - I_0 = I_{St}$, where the difference I_{St} should correspond to the signal intensity from the standard alone. In this way the ratio $I_0 : I_{St}$ can be found, serving as a convenient relative expression for comparing the signal intensity from different samples (I_0) with the absorption intensity of the permanent standard (I_{St}).

If the concentration of paramagnetic centres in the samples is to be calculated, it is necessary to 'calibrate' the standard signal. It must be remembered that the same amount of paramagnetic centres gives weaker absorption, when transferred from the axial part of the cavity. This is due to the fact that the intensity of the magnetic component of the microwave field decreases with increasing distance from the axis. The calibration is performed in the same way as that described for the measurement procedure. The only difference is that instead of a sample, the substance containing a known amount of paramagnetic centres is introduced into the cavity.

The possibility of placing standard and sample in the cavity is of special importance if materials having different dielectric properties are examined, since in this case the lowered Q-value affects signals from both standard and sample.

[1] The investigations were carried out during the author's tenure of a Fellowship of the Italian Government at the University of Palermo under the direction of Professor A. MONROY (Institute of Comparative Anatomy) and Professor U. PALMA (Institute of Experimental Physics), to whom the author is deeply indebted for their most valuable advice, stimulating suggestions, and for making accessible the modern equipment. The author wishes also to express his gratitude to all the members of the two Institutes, and in particular to Dr. L. BELLOMONTE and Dr. F. PERSICO for their very friendly helpfulness.

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The question to what degree the effects produced by the distortion of the microwave field in the cavity are significant requires separate treatment. It will be discussed elsewhere together with a more detailed description of the method. The results of preliminary tests, however, are encouraging and indicate that the 'magnet-rotation method', although comparatively simple, does not appear to be less exact than other techniques at present in use.

Riassunto. Si descrive un metodo che permette di misurare l'intensità di assorbimento ESR quando il campione standard e il campione su cui si esperimenta sono contemporamente presenti dentro la cavità risonante. Il campione standard viene situato in modo tale da rendere possibile l'eliminazione dell'assorbimento a cui esso da luogo, rotando il magnete attorno all'asse della cavità.

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Bovine Muscle Proteins
IV. Preparation of Myosin and Actomyosin

For our early studies of bovine myosin[1], several classical procedures[2–4] for the preparation of rabbit myosin were adapted. However, because of the size of the animals used, and the slaughtering techniques involved, muscle samples generally are not available for extraction until as much as 1 h after slaughter. During this period the pH of the muscle decreases much more than occurs in the short period (5–15 min) before extraction of rabbit myosin is begun; this pH drop can be compensated for by extraction with solutions of higher initial pH. Any further delay results in greatly decreased yield, for myosin loses extractability as the onset of rigor approaches (2–4 h after slaughter for lean beef muscles).

For rabbit myosin, an extract containing the myosin from 1 kg of muscle must be diluted to 35 l in order to precipitate the protein by the usual procedure[2–4] of lowering the ionic strength. To precipitate bovine myosin, the ionic strength must be lowered more by diluting the extract to 70 l; even then the protein is partially soluble, and so the yield is poor. Further losses occur when adenosinetriphosphate (ATP) is used to precipitate actomyosin. Such preparations also are not always free of turbidity. Moreover, bovine preparations using the procedure of SZENT-GYÖRGYI[3] usually give a product which is unsatisfactory, showing polydispersibility in the ultracentrifuge.

For our recent studies on the properties of bovine myosin[5,6], we have developed a more satisfactory method of preparation, based in part on DUBUISSON's finding[7] that actomyosin is precipitated at a lower concentration of ammonium sulfate than is myosin.

We have found that ammonium sulfate solutions can be used to extract myosin and actomyosin directly from the muscle tissue. From the extract obtained, first actomyosin, and then myosin, can be precipitated selectively by increase in ammonium sulfate content. With this procedure, the maximum volume of liquid needed is about 6 l/kg of meat. Further, the yield of the final purified protein generally is between 15 and 20 g/kg for myosin.

Bovine myosin can then be prepared in water-clear solutions up to 3% concentration in 0.6 *M* KCl (pH 6.5). From analysis of sedimentation patterns such as that shown in the Figure, the intrinsic sedimentation coefficient ($s_{20,w}$) of bovine myosin has been calculated to be 5.78 Svedberg units. Both the customary ATPase[5] and 5'-adenylate deaminase activities are present in myosin and the derived meromyosin[6] products. These procedures have also been applied to the isolation of rabbit and lamb myosins with comparable yields in both cases.

The procedure we have used is described below in detail as it has been applied to bovine muscle tissue obtained within 1 h after slaughter. The lean is separated quickly from visible fat and connective tissue, sliced into thin strips, chilled in ice and then ground in a pre-chilled Hamilton Beach[8] grinder.

Each 500 g of ground tissue is washed twice with 1 l of cold 0.05 *M* KCl solution; after each washing press out the liquid through cheese-cloth. Make a slurry of the meat residue with 1 l of cold 30%-saturated ammonium sulfate[9] solution (pre-adjusted with K_2HPO_4 to pH 6.8). After 10 min of gentle stirring, add another liter of the same solution, and squeeze quickly through cheesecloth. Adjust the pH between pH 6.5 and 7.0, and measure the volume of the extract. Because of the liquid in the original meat residue, the extract at this point contains about 25%-saturated ammonium sulfate. For each liter of extract, add 120 ml of 90%-saturated ammonium sulfate solution to raise the concentration to 32% of saturation. Using the GSA rotor with the Servall SS-3 centrifuge, separate out the actomyosin precipitate by centrifuging the mixture at 9000 × *g*, 10 min, at 4°C.

The myosin in the supernatant can be precipitated by carefully stirring in, for each liter of solution, 185 g of finely granulated ammonium sulfate, thereby bringing its concentration up to 60% of saturation. The preparation is again centrifuged as above; the sediment contains the myosin. For each 12 g (which is equivalent to 10 ml of 60%-saturated ammonium sulfate solution), add 10 ml of cold water, followed by an equal volume of cold 30%-saturated solution; adjust the pH between pH 6.5 and 7.0, if necessary.

Further purification is achieved by two or three reprecipitations, until the color of trace amounts of myoglobin is eliminated. After dissolving the myosin pellet with 5/6 vol of cold water, the solution at this point should

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