serum with saline and injected into paws of rats in the volume of 0·1 ml. The degree of inflammation was expressed as the difference in mm between the original dorsoplantar diameter of the paw and values attained 15 min after the injection of the tested solution (Fig. 3). The protein fraction containing most of the esterase activity produced also maximum inflammatory reactions.

The hypothetical enzyme plays possibly a significant role in the process of inflammation and experiments are planned to elucidate this role.

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Biosynthesis of norepinephrine and norsynephrine in the perfused rabbit heart

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CONSIDERABLE evidence has been obtained that confirms the postulated pathway by Blaschko[1] for the stepwise formation of norepinephrine. The decarboxylation of dopa and the β-hydroxylation of dopamine to form norepinephrine has recently been studied extensively *in vivo* and *in vitro*. Virtually nothing is known about the formation of dopa from tyrosine in mammalian enzyme systems, except that this reaction occurs in adrenals[2] and in certain sympathetic nerve tissues.[3] 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 norepinephrine.

In recent studies on β-hydroxylation of dopamine, it was shown that after infusion of rabbits with dopamine-^{14}C, high amounts of norepinephrine-^{14}C were found in the hearts.[4] The norepinephrine in the heart may have accumulated through uptake from the blood or through synthesis in the heart itself. The ability of the heart to form norepinephrine from various possible precursors was tested in the present study. The rabbit heart was perfused with dopamine-^{14}C, tyramine-^{14}C, and tyrosine-^{14}C, and the formation of catechols and β-hydroxylated compounds was investigated.

Rabbits were pretreated with 100 mg iproniazid/kg and killed 16 hr later. The thorax was immediately opened and the heart removed and perfused by the Langendorff technique with oxygenated (95% O_2, 5% CO_2) Krebs–Ringer bicarbonate solution at 38°. 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 that involves extraction into organic solvents, ion exchange, alumina and paper chromatography, and acetylation procedures.[5, 6]

The radioactivities in the β-hydroxylated products isolated from heart tissue after perfusion with the various precursors are shown in Table 1. It is evident that 1 to 3 per cent of the administered dopamine-^{14}C was converted to norepinephrine which was accumulated in the heart tissue. In the perfusate, unchanged dopamine-^{14}C and two O-methylated metabolites, 3-methoxytyramine and normetanephrine, were found (Table 2). No norepinephrine was detected in the perfusate.

After perfusion with tyramine-^{14}C, norsynephrine was isolated from the heart tissue (Table 1). Only negligible amounts of unchanged tyramine-^{14}C were found. The perfusate contained tyramine-^{14}C and other metabolites which have not been identified at the present time (Table 2). Neither dopamine-^{14}C nor norepinephrine-^{14}C has been detected in the heart tissue or in the perfusate after perfusion with tyramine-^{14}C.

TABLE 1. RADIOACTIVITY OF β-HYDROXYLATED PRODUCTS OBTAINED FROM ISOLATED RABBIT HEART PERFUSED WITH VARIOUS PRECURSORS

Perfused precursor			Perfusion Time	Product*			
Compound	Weight (mg)	cpm × 10^6	(mm)	Compound	Exp. 1 (cpm × 10^3/10 g tissue)	Exp. 2	Exp. 3
Dopamine	0·23	1·7	120	Norepinephrine	15	28	45
Tyramine	0·04	1·6	45†	Norsynephrine	25	41	55
Tyrosine	0·85	3·5	120	Norepinephrine	9·5	9·7	

* The radiochemical purity of the products was established by paper chromatography in two different solvent systems of the free compounds and acetylated derivatives.

† The radioactive compound was perfused for 15 min, and the perfusion was continued for the next 30 min.

TABLE 2. IDENTIFICATION OF METABOLITES FROM HEART TISSUE AND PERFUSATE AFTER PERFUSION OF THE RABBIT HEART WITH VARIOUS PRECURSORS

Perfused precursors	Metabolites	
	Heart tissue	Perfusate
Dopamine	Norepinephrine	3-Methoxytyramine normetanephrine
Tyramine	Norsynephrine	Unidentified metabolites
Tyrosine	Dopa Dopamine Norepinephrine	Dopa

In the experiments with tyrosine-^{14}C, the deproteinized heart tissue extract and the perfusate were chromatographed separately on alumina columns. The eluate from the alumina column contained the catechols whereas the effluent contained tyrosine and phenolic metabolites. The catechols were further purified on a Dowex-50 K^+ column which separates the catechol amino acids from the catecholamines. Further identification of the catechol amino acids and catecholamines formed during the perfusion was achieved by acetylation procedures and subsequently by paper chromatography. As Table 1 shows, after perfusion with tyrosine-^{14}C, norepinephrine-^{14}C was isolated from the heart tissue. Dopamine-^{14}C and dopa-^{14}C were also isolated from the heart tissue and dopa-^{14}C from the perfusate (Table 2). Whether tyramine or norsynephrine is formed after perfusion with tyrosine is now under investigation. Table 2 summarizes all the products isolated from the heart tissue and from the perfusate after perfusion with various precursors.

At the time this work was completed, similar independent findings on the formation of norepinephrine from dopamine in the isolated canine heart were reported.[7] It is therefore established that rabbit and canine hearts are capable of synthesizing norepinephrine from its immediate precursor, dopamine.

The conversion of tyramine to norsynephrine by adrenal and brain tissue slices was previously reported.[8] It was shown that the enzyme which converts dopamine to norepinephrine is not specific and that tyramine is a substrate of the same enzyme.[9] It may therefore be assumed that tyramine was converted in the isolated heart to norsynephrine by the same enzyme that formed norepinephrine from dopamine. It was recently reported that tyramine is converted *in vivo* to norepinephrine.[10] In the present studies a conversion of tyramine to norepinephrine could not be demonstrated, and therefore it seems unlikely that this reaction occurs in organs innervated by sympathetic nerves.

The present study shows that the heart is capable of performing all the enzymatic reactions necessary for the biosynthesis of norepinephrine:

tyrosine → dopa → dopamine → norepinephrine

The conversion of tyrosine to dopa may be the rate-limiting step in the biosynthesis of norepinephrine, and a detailed study of this enzymatic reaction is of great importance. The finding that tyrosine is hydroxylated to dopa whereas tyramine is not converted to dopamine in the isolated heart shows specificity of this reaction. It is of considerable interest now to investigate whether other phenolic amino acids are also converted by this system to the corresponding catechols. Preliminary studies show that *p*-fluoro-DL-phenylalanine inhibits the formation of catechols from tyrosine in the perfused heart. A more detailed study on the inhibition of catechol formation from tyrosine is in progress. Attempts are also now being made to isolate the enzyme from the heart in order to study the cofactor requirements and the substrate specificity.

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Added in proof: A recent publication by S. Spector, *et al.*, *Science* **139**, 1299 (1963), also describes the foundation of norepinephrine from tyrosine in the perfused heart.