No significant differences in the results obtained with the two gases were observed. For technical reasons the hydrogen appearance times were more accurately timed than those for oxygen, but for either gas appearance times are very brief and are of the same order of magnitude.

It is concluded from this study that oxygen and hydrogen can diffuse in detectable amounts directly from the alveoli through the walls of adjacent pulmonary arteries as large as 2 mm in diameter. Of several implications suggested, one of the most interesting is that the diffusion into the arterial walls plays some role in the control of the pulmonary circulation through changes produced in vasomotor activity of the pulmonary arteries.

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References and Notes

1. E. Weibel, *Z. Zellforsch.* **50**, 653 (1959).
2. L. C. Clark, *Trans. Am. Soc. Artificial Internal Organs*, **6**, 348 (1960).
3. L. C. Clark, *Circulation*, **22**, 949 (1960).
4. N. C. Staub, *Federation Proc.*, **20**, 107 (1961).

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Biosynthesis of Norepinephrine in Isolated Canine Heart

Abstract. *Radioactive dopamine was injected into the blood that was perfusing an isolated heart preparation. Analysis of the norepinephrine obtained from the heart 1 hour later demonstrated that it contained radioactivity. Between 1.4 and 10.8 percent of the norepinephrine present had been formed from the dopamine. The turnover of norepinephrine was substantially higher in the ventricles than in the atria.*

Although the pathway by which norepinephrine and epinephrine are synthesized was first suggested in 1938, it was not until the advent of radioisotopic techniques that this suggestion was confirmed for the adrenal medulla by a number of investigators (*1*). In vitro studies with minced tissue have shown that sympathetic nerves are also capable of synthesizing norepinephrine from either tyrosine or 3,4-dihydroxyphenylalanine (*2*). The heart, among other organs innervated by sympathetic nerves, can extract norepinephrine from the blood (*3*), but it is not known whether the heart is capable of synthesizing norepinephrine from its immediate precursor, dopamine. In our studies we administered radioactive dopamine to an isolated heart preparation and later measured the radioactivity which appeared in the norepinephrine isolated from the heart.

The canine heart preparation we used has already been described in detail (*4*). Oxygenated blood was pumped into the coronary circulation through a cannula inserted into the subclavian artery just proximal to the point at which the descending aorta was ligated. Both venae cavae and the azygous vein were ligated, and the coronary venous return was drained by gravity with a cannula in the right ventricle; the coronary venous blood was then returned to the oxygenator. The circuit of the oxygenator was filled with approximately 1000 ml of blood. Adrenalectomized dogs were used as donors because the levels of catecholamines in dogs subjected to hemorrhage are usually high. We tested the 3,4-dihydroxyphenylethylamine-1-C^{14} hydrobromide, or dopamine (supplied by New England Nuclear Corp.), for isotopic purity by paper chromatography. However, when 20 μc of dopamine were carried through the assay described below, a trace contaminant, the chromatogram of which resembled the chromatogram of norepinephrine, appeared. To eliminate this, the dopamine was further purified by adding 1 mg of nonradioactive norepinephrine and separating the radioactive dopamine by passing the mixture through a Dowex 50 (H^+) column (*5*).

The dopamine-C^{14} (17 μc, 3.10 to 3.26 μc/μmole), in 5 ml of an isotonic saline solution, was administered into the blood entering the coronary arteries during a 30-second period. Perfusion of the isolated heart was continued for the next 60 minutes, and then the heart was removed and frozen immediately in dry ice. This time interval was used because it was judged to be sufficiently long to permit formation of norepinephrine, but well within the time period during which the mechanical activity of the heart was sustained. In experiments 1 and 2 samples of the ventricles, in experiment 3 the entire heart, and in experiments 4, 5, and 6 both atria and ventricles were separated and homogenized with at least five times their volume of 5 percent trichloroacetic acid. The catecholamines in the extract were then adsorbed on aluminum oxide and eluted with 0.2*N* acetic acid by a modification of the procedure of Crout

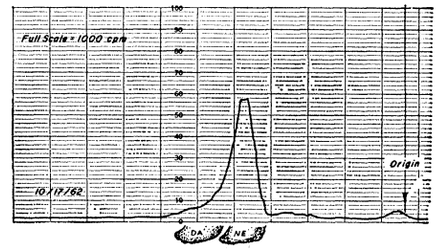

Fig. 1. Radiochromatogram of C^{14}-norepinephrine from isolated, perfused canine heart. (Whatman No. 1 paper; solvent: butanol, acetic acid, and water, 12:3:5). The chromatogram was developed with a ferricyanide spray and is shown together with the scan. The peak of radioactivity corresponds to the norepinephrine spot on the paper chromatogram. Minimal activity appears over the position of dopamine.

et al. (*6*). The eluate was lyophylized, and the residue, redissolved in 3 ml of 0.01*N* HCl, was passed through a column containing Dowex 50 X8 (H^+), 29 mm^2 by 45 mm, with 1.0*N* HCl as the eluent (*5*). Aliquots of the fraction of the eluate corresponding to norepinephrine were assayed for norepinephrine by a fluorometric method (*5*) and for radioactivity by liquid scintillation spectrometry. The isotopic purity of the norepinephrine fraction was demonstrated by ascending paper chromatography (see below). A minimum of 7 μg of norepinephrine was recovered from the chromatography on the Dowex 50 column in all experiments.

In all six experiments radioactivity was incorporated into norepinephrine, with the specific activities ranging from 0.045 to 0.334 μc/μmole (Table 1). In each of the last three experiments, in which the specific activity of norepinephrine in the ventricles was compared with that in the atria, the activity was approximately twice as great in the ventricles. In the four experiments in

Table 1. Specific activity of norepinephrine obtained from isolated canine hearts perfused for 1 hour with 20 μc of C^{14}-labeled dopamine.

Exp.	Specific activity (μc/μmole)			
	Dopamine	Norepinephrine		
		Whole heart	Ventricles	Atria
1	3.26		0.098	
2	3.26		.045	
3	3.10	0.148		
4	3.10	.214*	.236	0.122
5	3.10	.294*	.334	.174
6	3.10	.226*	.254	.138

* Specific activity calculated from the specific activities of the ventricles and atria analyzed individually.

which the entire heart was analyzed, it was possible to determine the total amount of radioactive norepinephrine formed. This value ranged from 0.110 to 0.161 μc per heart, and the fraction of the administered dopamine-C^{14} converted to norepinephrine ranged from 0.65 to 0.94 percent.

A radiochromatogram of the purified norepinephrine, shown in Fig. 1, demonstrates that the material is contaminated with only a small amount of radioactivity corresponding to the R_F of dopamine. To quantify the amount of this contamination more accurately, the specific activity of norepinephrine was determined before paper chromatography in one experiment and was found to be 0.24 μc/μmole. The area of the paper corresponding to the R_F of norepinephrine was then eluted, and the eluted radioactivity was adsorbed onto aluminum oxide. The specific activity of the aluminum oxide eluate was 0.22 μc/μmole. These results indicate that less than 10 percent of the radioactivity in the norepinephrine purified by the method employed in these experiments was due to contamination by dopamine.

The rate of synthesis of norepinephrine in these experiments may be estimated by relating the specific activity of the norepinephrine isolated to that of its precursor dopamine. If the neurotransmitter store were entirely replaced by norepinephrine newly formed from dopamine-C^{14}, its specific activity would be identical (100 percent) with that of the precursor. Actually, the specific activity of the isolated norepinephrine was found to range between 1.4 and 10.8 percent of the specific activity of the administered dopamine, indicating that between 1.4 and 10.8 percent of the norepinephrine in the heart after 1 hour's perfusion originated from the administered precursor. This fraction is a minimal estimate of the rate at which norepinephrine can be formed in the heart, being only a measure of the rate of formation from dopamine-C^{14}. The results of three experiments indicate that this fractional rate is substantially greater in the ventricles than in the atria (Table 1). The highest rate measured (10.8 percent per hour) may be sufficient for replacement of the entire norepinephrine store within a few hours. It is appreciated that these observations were made in isolated, perfused hearts and that the turnover is probably more rapid in intact animals, in which it may be conditioned by the rate of release of norepinephrine.

Our results show that dopamine is capable of acting as a precursor of norepinephrine in the heart. It remains to be determined whether synthesis of norepinephrine can be demonstrated from other potential precursors. Creveling *et al.* (*7*) have demonstrated that tyramine can serve as a precursor of norepinephrine in the whole rat, but three preliminary experiments with radioactive tyramine in the isolated canine heart preparation described herein have failed to demonstrate incorporation of its radioactivity into the norepinephrine isolated from the heart. It therefore appears unlikely that the whole rat has biosynthetic pathways for the formation of norepinephrine which are not present in the canine heart.

Our studies show that the heart can synthesize norepinephrine, and it need not be postulated that this organ is totally dependent upon extraction of norepinephrine from the blood to maintain its store. However, our observations do not provide evidence of the relative importance of synthesis and extraction in the maintenance of the neurotransmitter store in the intact animal.

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References and Notes

1. H. J. Schumann, in *Adrenergic Mechanisms* (Ciba Foundation Symposium), J. R. Vane, Ed. (Little, Brown, Boston, 1960), p. 6.
2. McC. Goodall and N. Kirschner, *Circulation* **17**, 336 (1958).
3. W. Raab and A. B. Gigee, *Circulation Res.* **3**, 553 (1955); L. G. Whitby, J. Axelrod, H. Weil-Malherbe, *J. Pharmacol. Exptl. Therap.* **132**, 193 (1961).
4. C. A. Chidsey, R. L. Kahler, L. L. Kelminson, E. Braunwald, *Circulation Res.*, in press.
5. A. Bertler, A. Carlsson, E. Rosengren, *Acta Physiol. Scand.* **44**, 273 (1958).
6. J. R. Crout, C. R. Creveling, S. Udenfriend, *J. Pharmacol. Exptl. Therap.* **132**, 269 (1961).
7. C. R. Creveling, M. Levitt, S. Udenfriend, *Life Sciences* **1**, 523, (1962).

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Stratigraphy of Beds I through IV, Olduvai Gorge, Tanganyika

Abstract. *Bed I at Olduvai Gorge is a conformable sequence of lava flows and varied sedimentary deposits that extend upward from a welded tuff overlying the Precambrian basement to the top of a widespread marker bed. Bed II is a sequence of lacustrine clays and laterally equivalent fluvial, eolian, and pyroclastic deposits. Bed III is made up of alluvial deposits and a laterally equivalent assemblage of fluvial, lacustrine, and eolian beds. Bed IV can be subdivided into a lower unit of fluvial clays, sandstones, and conglomerates, and an upper unit of eolian tuffs. The climate was relatively dry throughout much of the time that these beds were deposited, and semidesert or desert conditions may have prevailed at least twice. Tectonic movement seems to have taken place between the deposition of Beds III and IV.*

A stratigraphic and environmental framework more detailed than that of Reck (*1*) and Pickering (*2*) is presented here for the Pleistocene succession at Olduvai Gorge (Fig. 1), which contains hominid remains of great antiquity (*3*) and an unsurpassed sequence of Paleolithic culture levels (*4, 5*). The need for geological information about the succession at Olduvai became clear in recent controversies about K-Ar dates and the geologic histories of Beds I and II (*6, 7*). At least some of this argument would not have arisen if the geology had been properly understood. This paper summarizes the principal results of 8 weeks of geologic field work at Olduvai Gorge during the summer of 1962 (*8*). Field work has been supplemented by extensive microscopic and x-ray study of the rocks. The stratigraphic synthesis of Fig. 2 is based on approximately 50 measured sections and the lateral tracing of key horizons. This report is primarily intended to clarify the major stratigraphic relationships; the geology will be described more fully in a subsequent paper.

The succession in Olduvai Gorge was divided by Reck (*1*) into a basal series of basalt flows and mappable units termed Bed I, Bed II, Bed III, Bed IV, and Bed V. Bed V overlies the older beds with pronounced angular unconformity and will not be considered further. The subdivision and nomenclature of Reck are used here, with few modifications.

The basalts comprise a lower flow, 35 to 40 feet thick, which has a typical aa surface structure, and an overlying series of thinner, pahoehoe flows. The aa flow is a biotite-bearing olivine