portional to the difference between the signals from the transducers are also needed. The most troublesome problem has been the elimination of any factor tending to modify the dynamic characteristics of one lumen with respect to the other. Very fine bubbles in the system that do not visibly affect the pressure tracings can so change the characteristics of one lumen that the differential pressure tracing is seriously degraded.

It is believed that the method provides a means of measuring the instantaneous linear velocity in any vessel having a significant rate of flow. Volume flow per beat, such as the stroke volume in the pulmonary artery, can be calculated from the differential pressure curves if some estimate of the diameter of the pulmonary artery is available. A square-front velocity distribution is assumed in such calculations because of the pulsatile nature of the flow (*3*). Values obtained can be checked by standard Fick output studies.

The method appears to have applicability in normal and pathological situations, in steady and nonsteady states, in animals and in man.

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References and Notes

1. This work was supported in part by the New York Heart Association.
2. I. G. Baxter and J. W. Pearce, *J. Physiol. (London)* 115, 410 (1951).
3. D. L. Fry, A. J. Mallos, A. G. T. Casper, *Circulation Research* 4, 627 (1956).

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O-methylation, the Principal Route of Metabolism of Epinephrine in Man

Abstract. Evidence is presented which indicates that the principal pathway of metabolism of epinephrine in man is O-methylation to metanephrine. The extent of the conversion to metanephrine, a physiologically inactive compound, indicates that the enzyme responsible for this reaction, catechol-O-methyl transferase, is the enzyme mainly involved in the termination of action of epinephrine in man.

For many years monoamine oxidase has been considered to be the primary enzyme in the metabolism of epinephrine. However, recent work in this laboratory has demonstrated that epinephrine and norepinephrine are transformed in the rat mainly by O-methylation to yield metanephrine (*m*-O-methyl-epinephrine) and normetanephrine (*m*-O-methyl-norepinephrine) (*1*). This reaction has been shown to be catalyzed by the enzyme, catechol-O-methyl transferase (*1, 2*). It was of interest, therefore, to determine the extent of O-methylation of epinephrine in man.

Table 1. Fate of β-H^3-epinephrine in man.

Subject	Radioactivity excreted in urine				
	As percent of administered dose	Percent of urinary radioactivity present as:			
		Metanephrine			VMA†
		Free	Conjugated	Total*	
		β-H^3-epinephrine administered			
R.C.	95	13	46	59	30
D.K.	95	14	38	52	37
R.B.	88	11	36	47	35
D.H.	73	10	49	59	40
		β-H^3-metanephrine administered			
W.N.	92	16	39	55	24
K.S.	88	11	33	46	25

* Metanephrine found after heating in 1*N* HCl for 30 minutes at 100°C. Conjugated metanephrine represents the difference between free compound and total. A small amount of metanephrine glucosiduronic acid (6 percent) is included in the conjugated fraction.
† 3-Methoxy-4-hydroxymandelic acid (vanillylmandelic acid). This fraction also contains an unidentified metabolite which represents about 5 percent of the total radioactivity in the urine.

One milligram of *d,l*-β-H^3-epinephrine bitartrate (267 μc) was given intravenously over 45 minutes to four normal young male subjects, and the urine was collected for 48 hours. During this time, 73 to 95 percent of the administered radioactivity appeared in the urine (*3*). Metanephrine was extracted into isoamyl alcohol at *p*H 10, and the extract was assayed for radioactivity (*3*). On Whatman No. 1 paper the extracted radioactive material exhibited a single peak having the same R_F as authentic metanephrine (*1*) after chromatography in butanol–acetic acid–water (8:2:2) and isopropanol–5 percent ammonia (8:2). The 3-methoxy-4-hydroxymandelic acid was extracted at *p*H 1 into isoamyl alcohol. The extract was shaken with 5 percent sodium bicarbonate; the aqueous layer was re-extracted into isoamyl alcohol at *p*H 1, which was assayed for radioactivity (*3*). After chromatography, using the systems described above, the final extract showed a major peak having the same R_F as authentic 3-methoxy-4-hydroxymandelic acid and a small peak representing an unidentified compound present to the extent of 5 percent of the total radioactivity in the urine. All determinations of radioactivity were made by using internal standards and were corrected for the partitions of the O-methylated metabolites in the two-phase systems described above.

Free and conjugated metanephrine (Table 1) accounted for 54 percent of the radioactivity in the urine. These results differ from those published by others (*4*) in which little metanephrine was found in the urine following the infusion of C^{14}-epinephrine to man. We also found that 3-methoxy-4-hydroxymandelic acid accounted for 36 percent of the radioactivity in the urine (Table 1). Armstrong and McMillan (*5*) have reported the latter compound to be a major metabolite of norepinephrine.

To examine the relative importance of deamination in the metabolism of epinephrine, the metabolism of its major product, metanephrine, was studied.

About 0.7 mg of β-H^3-metanephrine (*6*) was administered intravenously to two normal young males over 45 minutes, and urine was collected for 48 hours. In contrast to the marked physiological and psychological effects produced by β-H^3-epinephrine, no detectable effects accompanied infusion of β-H^3-metanephrine. As in the case of β-H^3-epinephrine, 88 to 92 percent of the radioactivity was excreted in the urine. Essentially the same fraction of the administered radioactivity was excreted as metanephrine (free and conjugated) after administration of β-H^3-metanephrine as was found after administration of β-H^3-epinephrine (Table 1). A considerable proportion of administered metanephrine was deaminated and excreted as 3-methoxy-4-hydroxymandelic acid, indicating that a significant fraction of the metanephrine arising from epinephrine is further metabolized to 3-methoxy-4-hydroxymandelic acid.

These results indicate that the role of monoamine oxidase in epinephrine metabolism is mainly in the deamination of metanephrine. This would explain the observations that iproniazid, an inhibitor of monoamine oxidase, does not prolong the physiological actions of epinephrine in vivo (*7*).

From our results it can be concluded that the principal pathway of metabolism of epinephrine in man is its O-methylation to metanephrine, which in turn is conjugated and deaminated.

This, together with the lack of physiological and psychological action of 3-O-methyl catechol amines observed by others with normetanephrine (*8*) and by us with metanephrine, points to catechol-O-methyl transferase as the principal enzyme for the inactivation of epinephrine.

Note added in proof: Since this communication was submitted for publication, a paper by Kirshner *et al.* (*9*) has appeared which reports that 47 percent of the radioactivity in the urine is "3-methyl-O-adrenaline" (metanephrine), free and conjugated, following the administration of *d,l*-adrenaline-2-C^{14} to man. This finding differs from that reported previously by these workers (*4*).

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References and Notes

1. J. Axelrod, *Science* 126, 400 (1957); J. Axelrod *et al., Biochim. et Biophys. Acta* 27, 210 (1958); J. Axelrod, S. Senoh, B. Witkop, *J. Biol. Chem.*, in press.
2. J. Axelrod and R. Tomchick, *J. Biol. Chem.*, in press.
3. Radioactivity was determined by a modification of the method of G. T. Okita *et al.* [*Nucleonics* 14, No. X, 76 (1956)]. The sample (0.1 ml of urine or 4 ml of isoamyl alcohol extract) was added to a mixture of 4 ml of ethanol and 10 ml of 0.4 percent 2,5-diphenyloxazole and 0.01 percent β-bis[2-(phenyloxazolyl)]benzene in toluene and counted in a liquid scintillation spectrometer.
4. O. Resnick *et al., Science* 127, 1116 (1958); McC. Goodall, L. Rosen, N. Kirshner, *Federation Proc.* 17, 56 (1958).
5. M. D. Armstrong, A. McMillan, K. N. F. Shaw, *Biochim. et Biophys. Acta* 25, 422 (1957).
6. This compound was prepared enzymatically by the O-methylation of β-H^3-epinephrine (*2*).
7. S. J. Corne and J. D. P. Graham, *J. Physiol. (London)* 135, 339 (1957); E. C. Griesemer *et al., Proc. Soc. Exptl. Biol. Med.* 84, 699 (1953).
8. E. V. Evarts *et al., Proc. Soc. Exptl. Biol. Med.* 98, 74 (1958).
9. N. Kirshner, McC. Goodall, L. Rosen, *Proc. Soc. Exptl. Biol. Med.* 98, 627 (1958).

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Evidence That the Mammalian Nephron Functions as a Countercurrent Multiplier System

Abstract. Fluid collected by micropuncture from the bend of the loop of Henle in the concentrating hamster kidney had the same osmotic pressure as fluid from a collecting duct at the same level, while that from the distal convolution was more dilute. This indicates that the tubular fluid is first concentrated, then diluted, before its final concentration.

Hargitay and Kuhn (*1*), in 1951, introduced a new and revolutionary concept for the mechanism of urine concentration in the mammalian kidney based on the premise that the loop of Henle acts as a countercurrent multiplier system. The loop of Henle mechanism was thought to make the interstitium of the medulla hyperosmotic, which in turn caused diffusion of water out of the collecting ducts and concentration of the urine. The great theoretical advantage of this mechanism is that at no level in the kidney need there be large osmotic gradients maintained by tubular structures only one cell layer thick.

The original experimental observations in support of this theory were those of Wirz, Hargitay, and Kuhn (*2*), who concluded from cryoscopic studies of slices from concentrating rat kidneys that the osmotic pressure was identical for all adjacent tubular structures at any level in the kidney, and that there was a steadily increasing osmotic gradient from the cortex, which was isosmotic with plasma, to the tip of the papilla. Recent analyses of distal tubular fluid by Wirz (*3*) and ourselves (*4*) demonstrate that fluid in all adjacent tubules does not have the same osmolality, invalidating this aspect of the original data which presumably was due to postmortem diffusion. This does not, however, invalidate the theory of increasing osmotic gradient from cortex to papilla. This report (*5*) presents the results of osmolality determinations on fluid collected directly from the loops of Henle of hamsters.

The papilla of the hamster's kidney, which extends into the upper portion of the ureter, was exposed in anesthetized animals. When observed microscopically, vasa recta and collecting ducts were readily apparent. Under proper illumination, segments of narrow tubular structures filled with clear fluid were sometimes visible. When punctured with a micropipette and filled with large amounts of dye, they were seen to form typical hairpin loops without anastomosis. On occasion it was subsequently possible to macerate the kidney and to follow the injected loop by microdissection to the proximal and distal convolutions of a juxtamedullary nephron, proving beyond doubt that the structure punctured in the papilla was a loop of Henle. In order to exclude the possibility that the sample was plasma from a vas rectum without red cells or anastomosis, it was sufficient to demonstrate that the sample contained little or no protein by qualitatively testing it with heat or trichloroacetic acid. Fluid was also collected from adjacent collecting ducts at the same level. Osmolality was determined by the microcryoscopic method of Ramsay and Brown (*6*).

The results of four typical analyses of fluid from the bends of the loops and collecting ducts are shown in Table 1. The osmolalities were the same or nearly so and were much higher than the osmolality of plasma from the inferior vena cava. Fluid from cortical segments of proximal tubules and late distal convolutions was isosmotic and from early distal convolutions, hypo-osmotic to plasma.

Table 1. Osmolality of fluid from the loops of Henle and collecting ducts and of plasma from the inferior vena cava.

Hamster No.	Osmolality (milliosmoles per kilogram of water)		
	Loop of Henle	Collecting duct	Plasma
1	1391	1402	308
2	725	720	336
3	1270	1206	325
4	453	453	

These results are highly consistent with the hypothesis of Hargitay and Kuhn (*1*) that the mammalian nephron functions as a countercurrent multiplier system to concentrate the urine. The details of the mechanism appear to be somewhat different from those first proposed (*7*).

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References and Notes

1. B. Hargitay and W. Kuhn, *Z. Elektrochem.* 55, 539 (1951).
2. H. Wirz, B. Hargitay, W. Kuhn, *Helv. physiol. et pharmacol. acta* 9, 196 (1951).
3. H. Wirz, *ibid.* 14, 353 (1956).
4. C. W. Gottschalk and M. Mylle, *Federation Proc.* 17, 58 (1958).
5. This work was supported by grants from the American Heart Association and the U.S. Public Health Service (H-2334-C). This work was done during the tenure by one of us (C.W.G.) of an established investigatorship of the American Heart Association.
6. J. A. Ramsay and R. H. J. Brown, *J. Sci. Instr.* 32, 372 (1955).
7. A detailed account of these and other studies is in preparation.

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A System of Names for Binary Numbers

Abstract. A nomenclature is proposed for the binary number system to permit expression of binary numbers in words and to encourage visualization of magnitudes expressed in binary notation without recourse to decimal translation.

Our everyday lives impinge increasingly on systems in which binary numerical notation is encountered—for example, computers, data-processing systems, accounting systems, counting devices, logical circuitry, communication, and instrument systems generally. Familiarity with binary notation and some ability to think in terms of binary arithmetic are necessary for all scientists and engineers and are desirable for any well-informed