duced by the pulmonary tissues can be calculated from

$$\dot{V}_{T_{CO_2}} = \dot{V}_{CO_2} - \dot{V}_{E_K}\left[\frac{C_{\bar{v}CO_2} - C_{aCO_2}}{C_{\bar{v}_K} - C_{a_K}}\right] \quad (8)$$

where the symbols correspond to those defined for oxygen. And, finally, the respiratory quotient (R_T) of the tissues can be calculated by dividing Eq. 7 into Eq. 8.

$$R_T = \frac{\dot{V}_{CO_2}(C_{\bar{v}_K} - C_{a_K}) - \dot{V}_{E_K}(C_{\bar{v}CO_2} - C_{aCO_2})}{\dot{V}_{O_2}(C_{\bar{v}_K} - C_{a_K}) - \dot{V}_{E_K}(C_{aO_2} - C_{\bar{v}O_2})}$$

In essence, this approach consists of simultaneously applying the Fick principle with two different gases. One of these, oxygen, is metabolized, while the other, an inert gas, is not.

The success of this approach hinges on the degree to which a steady-state can be approximated, particularly in regard to the equilibration of the gas with the tissues. If equilibrium cannot be achieved, $\dot{Q}_R$ can still be estimated by substituting the rate of infusion for $\dot{V}_{E_K}$ in the last four equations, and by using the gas concentration in arterial blood as an estimate of that in the blood draining from the tissues into the right side of the heart. Results from this method with the inert gas Kr^{85} have been more reproducible than those obtained with dye. But whether the errors will be sufficiently minimized to assure the applicability of the technique to patients free of pulmonary infections is not yet known.

If the utility of the approach can be established, the method might provide information on several disputed points. For example, the role of metabolism in the genesis of pulmonary diseases might be studied, the effect of therapy on the metabolism of pulmonary lesions might be investigated, and the participation of the pulmonary tissues in diseases of metabolism, such as hyperthyroidism, might be explored (*7*).

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References and Notes

1. H. A. Krebs, in *Handbuch der Biochemie des Menschen und der Tiere,* Oppenheimer, Ed. (Fischer, Jena, 1933) [cited in "Handbook of respiration," *Wright Air Development Center Tech. Rept. 58-352* (August 1958), p. 291].
2. H. W. Fritts, Jr., P. Harris, C. A. Chidsey III, R. H. Clauss, A. Cournand, J. *Appl. Physiol.* **11**, 362 (1957).
3. ———, *Circulation,* in press.
4. G. S. Dawes, J. C. Mott, J. G. Widdicombe, *J. Physiol. London* **126**, 38 (1954); G. V. R. Born, G. S. Dawes, J. C. Mott, J. G. Widdicombe, *Cold Spring Harbor Symposia Quant. Biol.* **19**, 102 (1954).
5. A. Carlyle, *J. Physiol. London* **107**, 355 (1948).
6. D. E. Gregg, *Physiol. Revs.* **26**, 28 (1946).
7. This investigation was supported by a research grant, H-2001 (C), from the National Heart Institute, U.S. Public Health Service, Bethesda, Md.

12 December 1960

Uptake of Tritium-Labeled Norepinephrine in Brain and Other Tissues of Cat in vitro

Abstract. Slices of cat cerebral cortex, heart, and spleen that have been incubated in media containing approximately 5 to 25 mμg of *dl*-norepinephrine-7H^3 per milliliter contain levels of isotopic amine greater than those in the medium. The effects of norepinephrine concentration, reserpine, and ouabain on the uptake suggest that the amine enters cells both by diffusion and by a concentrating mechanism that is saturated at low levels of norepinephrine. The drugs inhibit the latter.

Hughes and Brodie (*1*) have presented evidence that active transport of 5-hydroxytryptamine is an important factor in maintaining the high concentrations of this amine in blood platelets. The present studies were undertaken to determine whether a similar mechanism contributes to the relatively high levels of catecholamines maintained in parts of the brain and in sympathetically innervated organs (*2*).

Tissue slices were incubated in Krebs bicarbonate medium containing 5 mμg of *dl*-norepinephrine-7H^3 per milliliter. The concentration of isotope in cat cerebral cortex (Fig. 1) rose to several times that in the medium. Uptake curves for slices of hypothalamus, cerebellum, heart, and spleen were very similar to those in Fig. 1. Liver and muscle, however, did not accumulate concentrations of norepinephrine significantly greater than that in the medium. Approximately 75 percent of the radioactivity in cortex and heart after 1 hour of incubation was accounted for as unchanged norepinephrine by column chromatography of the contents of pooled slices (*3*). Chromatographic analysis of the incubation media indicated that 70 percent of the metabolites produced by cortical slices were 3,4-dihydroxy or 3-methoxy-4-hydroxymandelic acid. In the heart media the O-methyl ether of norepinephrine accounted for 60 percent of the metabolites.

In order to determine the effect of norepinephrine concentration on the rate of uptake, tissue levels of labeled norepinephrine were measured after 20 minutes of incubation, during which time the uptake is approximately linear. The ratio of tissue concentration of isotope to concentration in the medium was maximal in cortex and heart at levels between 5 and 25 mμg/ml. At higher levels the tissue concentration approached that of the medium. These data, which resemble those obtained with epinephrine (*4*), suggest that catecholamines, like 5-hydroxytryptamine in platelets (*1*), enter nervous tissue in two ways. At low levels of norepinephrine most of the inward flux would occur by a concentrating mechanism involving a carrier, presumably enzymatic. The data of Table 1 would be consistent with the saturation of such a carrier at norepinephrine levels of about 25 mμg/ml above which the rate of entry by this mechanism could not be further increased.

The second component of the inward flux would be a simple diffusion by which the internal concentration of the amine should gradually approach the external one. The rate of this process, however, should not be subject to an upper limit since diffusion rates vary linearly with concentration. Thus the limited quantity entering in a given time by the concentrating mechanism should become a negligible fraction of the much larger amounts forced through the diffusion barrier by very high external concentrations, and the ratios of Table 1 would be expected to approach unity.

Uptake of norepinephrine by the concentrating mechanism could represent either chemical binding to tissue components or active transport by an energy-requiring pump of low capacity. That it may be the latter is suggested by the marked inhibition of the inward flux by ouabain (Fig. 1), since cardiac

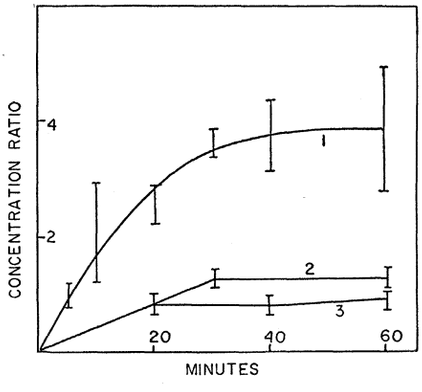

Fig. 1. Ratio of concentration of radioactivity in cat cerebral cortex slices to that in a Krebs bicarbonate medium containing 5 μg of *dl*-norepinephrine-7H^3 per milliliter. Curve 1, control. Curve 2, 24 hours after reserpine (3 mg/kg) was injected intraperitoneally. Curve 3, slices from normal cats incubated in media containing $10^{-5}M$ ouabain. Vertical bars represent standard deviation of values obtained with tissues from eight cats.

Table 1. Ratio of concentration of radioactivity in cat cerebral cortex to that in Krebs bicarbonate medium after incubation of slices for 20 minutes at 37°C with *dl*-norepinephrine-7H³.

Concentration (mμg/ml)	Ratios in	
	Normal medium	$10^{-6}M$ reserpine
1	0.63	0.58
5	2.28	1.43
25	1.85	1.35
100	1.63	1.50
200	1.17	0.98

glycosides are known to block the utilization of metabolic energy for the active transport of a number of substances (*5*). Reserpine is reported to block the active transport of 5-hydroxytryptamine in platelets (*1*). In cortex slices from reserpinized animals (Fig. 1), as well as in those exposed to reserpine in vitro (Table 1), the tissue concentration of norepinephrine after incubation was reduced. Reserpine apparently blocks the concentrating mechanism since at the higher concentrations of norepinephrine where inward flux is primarily by diffusion the effect of the drug was considerably lessened.

In the presence of the sulfhydryl inhibitors, iodoacetate ($10^{-3}M$), and *p*-chloromercuribenzoate ($5 \times 10^{-6}M$), norepinephrine uptake by cortex slices was reduced to 78 and 50 percent of control values. Dinitrophenol ($10^{-3}M$) reduced uptake by 25 percent. Fluoride and azide were without effect. Slices warmed at 55°C for 10 minutes in isotonic saline were unable to concentrate isotopic norepinephrine when incubated under the usual conditions.

Although isotope was more concentrated in tissue than in the medium after incubation, the concentration ratios were much lower than would be expected if there were complete isotopic exchange between extracellular and endogenous norepinephrine. Complete exchange between the radioactive norepinephrine in 3 ml of a solution containing 5 mμg/ml and the endogenous norepinephrine in a 100-mg slice of cortex (approximately 0.04μg) (*2*) should yield a ratio of isotope concentration in tissue to that in the medium of about 75. Since experimentally this ratio has never exceeded 5.8 after 1 hour of incubation, the isotopic norepinephrine must exchange slowly, if at all, with the amine in intracellular storage sites. In adrenal medulla (*6*), splenic nerve (*7*), and perhaps in nervous tissue generally (*8*) these storage sites appear to be cytoplasmic granules. Adrenal medulla was unusual in that slices of this tissue did not concentrate labeled norepinephrine to levels significantly greater than in the medium. This result is in keeping with Hillarp's observation (*9*) that isotopic epinephrine did not exchange with the epinephrine in isolated adrenal medullary granules, but seems surprising in view of the reported accumulation of isotopic norepinephrine in the rat adrenal after intravenous injection of small amounts (*10*).

It is possible that the concentrating mechanism delivers norepinephrine to an intracellular pool distinct from the particulate sites where much of the amine appears to be stored. Recent results of Burn and Rand would be consistent with the existence of such a pool (*11*).

A number of drugs that sensitize adrenergic organs to administered catecholamines inhibit uptake (*12*), by the concentrating mechanism observed here. It is possible that the latter represents a means for terminating the biological effect of catecholamines.

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References and Notes

1. F. B. Hughes and B. B. Brodie, *J. Pharmacol. Exptl. Therap.* **127**, 96 (1959).
2. M. Vogt, *J. Physiol. London* **123**, 451 (1954).
3. H. J. Dengler, *Arch. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's* **231**, 373 (1957).
4. B. B. Brodie, H. J. Dengler, E. Titus, C. W. M. Wilson, *J. Physiol. London*, in press.
5. S. Hajdu and E. Leonard, *Pharmacol. Revs.* **11**, 173 (195 → J. Wolff, *Biochim. et Biophys. Acta* **38**, 316 (1960).
6. N-A. Hillarp and B. Nilson, *Acta Physiol. Scand.* **32**, 11 (1954).
7. U. S. von Euler, *ibid.* **43**, 155 (1958).
→ H. Weil-Malherbe and A. D. Bone, *Nature* **180**, 1050 (1957).
9. N-A. Hillarp, *Acta Physiol. Scand.* **47**, 271 (1959).
→ L. G. Whitby, J. Hertting, J. Axelrod, *Nature* **187**, 605 (1960).
11. J. H. Burn and M. J. Rand, *J. Physiol. London* **144**, 314 (1958); *Brit. J. Pharmacol.* **15**, 47 (1960).
12. H. J. Dengler, H. E. Spiegel, E. Titus, unpublished observations.

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16 October 1960

Vessels in Roots of Marsilea

Abstract. The occurrence of vessel members in the roots and the possible occurrence of sieve-tube members in the rhizome is reported for the first time in the heterosporous fern *Marsilea quadrifolia*. This discovery adds a new instance of parallel evolution of vessels in vascular plants.

Vessel members, although highly characteristic of the flowering plants, occur also in other widely divergent groups of vascular plants (*1*) including the Selaginellales (*Selaginella*), the Equisetales [*Equisetum* (*2*)], the Genetales (*Gnetum, Welwitschia,* and *Ephedra*), and the Filicales [in which they have previously been reported only in *Pteridium* (*3*)]. It is my purpose herein to record for the first time their occurrence in the roots of the heterosporous fern, *Marsilea quadrifolia*. This discovery is of unusual interest as another example of parallel evolution in vascular tissue.

The vessel members of *Marsilea* have distinct end walls and are superposed in vertical columns (Fig. 1*c*). The end walls vary from steeply oblique with scalariform perforations through a series of intermediate forms (Fig. 1*a* and *b*) to transverse end walls with a simple perforation. Often a single vessel member will show both extremes —the steep scalariform perforated wall at one end, and the transverse simply perforated wall at the other. The individual vessel members average 3.6 mm in length and 27.4 μ in width. Reduced scalariform pitting occurs on the lateral walls.

A test was made to determine whether particles of India ink would pass through these cells (*4*). The ends of decapitated roots were immersed in ink, and it was found that the ink particles pass readily throughout their length. As these particles are too large to pass through pit membranes, it was concluded that the conducting cells must be true vessel members. It is significant that no ink particles were conducted into the rhizome where the conducting elements were found to be tracheids.

An examination of two other species of *Marsilea* (*M. drummondii* and *M. hirsuta*) revealed that they also possess vessels in their roots, but, as in *M. quadrifolia*, they lack vessels in the rhizome and petiole. Additional species of *Marsilea* are currently under investigation.

No vessels were observed in any of the organs of species in the two other genera of the Marsileaceae, *Regnellidium* and *Pilularia*. Likewise, none were found in species of *Salvinia* and *Azolla* of the other heterosporous fern family, Salviniaceae.

It is interesting that in the course of this survey, cells with the appearance of true sieve-tube members were observed in the phloem of *Marsilea*. These cells, which are very long, have sieve areas restricted to oblique end walls, and only irregularly scattered simple pits have been observed on the lateral walls. They form continuous series of cells in the internodal regions of the rhizome. Peculiarly, the sieve-tube members are confined to the rhizome, which has only tracheids in the xylem. The roots, which have vessels in the xylem, have only typical sieve cells in the phloem. If these cells