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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## 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

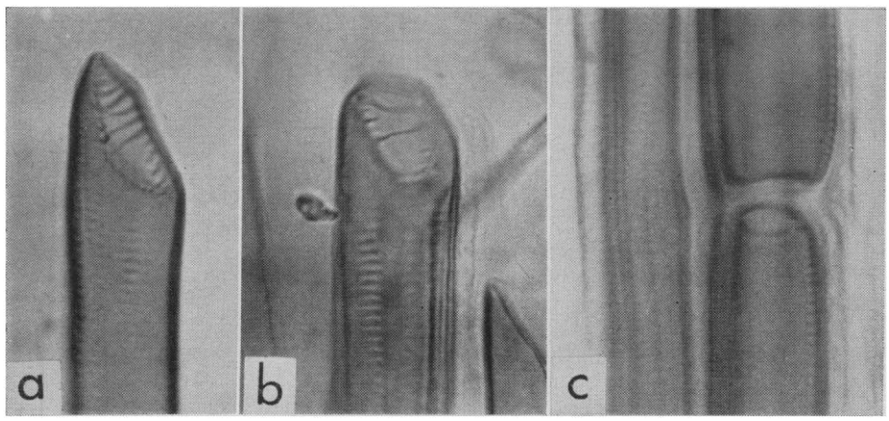


Fig. 1. Vessel members of *Marsilea quadrifolia*. Vessel members wtih scalariform-perforated end walls (*a, b*). The articulation of two vessel members with transverse, simple-perforated end walls (*c*).

are, indeed, sieve-tube members, their occurrence in *Marsilea* will be, as far as is known, unique within the Filicales and the only known occurrence outside of the angiosperms.

On the basis of frond form, *Marsilea* is generally regarded as the most primitive genus of the Marsileaceae. On the basis of sporangial type, however, *Marsilea* is evidently the most specialized (*5*). The evidence from the vascular tissue tends to support the latter.

The vessels of the bracken fern, *Pteridium*, are very different from those of *Marsilea*. They are located in the rhizome, petiole, and root and are not confined solely to the roots, as in *Marsilea*. Furthermore, the vessels of *Pteridium* possess members with only the scalariform type of perforation plate. Pits on the lateral walls are typically scalariform, whereas those of *Marsilea*, although scalariform, are relatively smaller and more widely spaced. In addition, vessel members in *Pteridium* are much shorter than those of *Marsilea*.

Both *Marsilea* and *Pteridium* are members of families considered to be taxonomically advanced. The heterosporous family Marsileaceae is generally considered more highly advanced than the Pteridaceae. The vessel members of *Marsilea*, on the basis of characters used to determine degree of specialization in angiosperms, are, with the exception of their length, more highly specialized than those in *Pteridium*.

The discovery of vessel members in *Marsilea* demonstrates once again the homoplastic development of these structures in taxonomically widely separated plants. In view of their occurrence in these distantly related genera, and the differences in morphology and location in the plant, it is probable that the vessels have originated independently at least twice in the ferns—in the terrestrial *Pteridium* and in the aquatic *Marsilea*. This parallels the situation that seems to have occurred in the angiosperms (*6*).

Histological and developmental studies will supply detailed information concerning the nature and ontogeny of the vessel and supposed sieve-tube elements in *Marsilea*. A statistical study of the tracheary elements of the ferns in progress will provide a broader basis for a more critical interpretation of the significance of these cell types in the Filicineae (*7*).

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## X-rays Affect the Incorporation of 5-Iododeoxyuridine into Deoxyribonucleic Acid

*Abstract.* When labeled with iodine-131, 5-iododeoxyuridine, an analogue of thymidine, is useful in estimating the effect of x-radiation on deoxyribonucleic acid metabolism. Although this compound is readily incorporated into deoxyribonucleic acid in the absence of ionizing radiation, we find that whole-body exposure to as little as 10 r will significantly inhibit its incorporation.

The debilitating effects of x-rays to higher animals are usually the delayed consequences of the inhibition of multiplication or death of cells in certain normally proliferative tissues. Tracer studies in turn suggest that the damage to proliferating cells can be correlated with altered metabolism of their genetic material, deoxyribonucleic acid (DNA). Tritiated-thymidine, while an excellent measure of DNA metabolism (*1*), is difficult to assay because of the weak energy of tritium's $\beta$-radiation; whereas an iodinated analogue would permit easy assay of whole tissues or animals by the gamma rays of radioiodine. Therefore, the present technique was developed, utilizing the thymidine analogue, iododeoxyuridine (*2*), which can be incorporated in place of thymidine into DNA (*3*). For this purpose the iododeoxyuridine was prepared labeled with $I^{131}$ by iodination of deoxyuridine as described by Prusoff (*2*) [but with the addition of radioactive iodine ($I^{131}$) to the reaction mixture] and purified by four recrystallizations from water.

Groups of Swiss albino mice, 6 to 10 weeks old, were exposed to 250 kv (peak) x-rays filtered through 0.5 mm of copper and 1 mm of aluminum. The rate of irradiation was 50 to 52 r/min, different doses being attained by varying the duration of exposure. At a variable time interval before the onset or after the cessation of irradiation, each mouse received a single intraperitoneal injection of 0.1 $\mu$mole of the labeled compound having a specific activity of approximately 10 $\mu$c/$\mu$mole; the controls were unirradiated litter mates, which had been similarly injected. All mice were given 0.1 percent NaI in their drinking water for at least 24 hours before injection with labeled iododeoxyuridine to prevent retention in the thyroid of radio-iodide, the major catabolic product.

The radioactivity of each mouse was counted periodically in a large well-type crystal scintillator. Figure 1 illustrates the average radioactivity retained (plotted logarithmically) by groups of three to five mice as a function of time after injection. In both irradiated and unirradiated animals the initial rapid disappearance of radioactivity was followed by a slow phase (from about the 10th to the 40th hour) in which the retained amount of activity remained essentially constant and can be attributed to label in metabolically stable DNA within new cells. The more rapid loss of radioactivity after 40 hours undoubtedly reflects the death of labeled cells. Chemical data in support of this interpretation are as follows.

At intervals, two or three mice from a group were killed, and their organs were either assayed for radioactivity directly or for activity insoluble in 10 percent trichloroacetic acid after homogenization, precipitation with this reagent, and washing until the supernatant contained less than 2 percent of the radioactivity of the precipitated