interest as a simple laboratory technique for the production of tracer C^{13}. The possible application of the high-cut method to the enrichment of other isotopes is being considered.

RICHARD B. BERNSTEIN
Chemistry Department,
University of Michigan, Ann Arbor

References and Notes

1. C. R. McKinney *et al., Rev. Sci. Instr.* 21, 724 (1950).
2. M. Dole, *Chem. Revs.* 51, 266 (1952).
3. For background on the influence of isotope effects on tracer studies, see J. Bigeleisen, *Science* 110, 14 (1949).
4. This research received financial support from the U.S. Atomic Energy Commission and the Michigan-Memorial Phoenix Project. I am indebted to the Alfred P. Sloan Foundation for a fellowship which made this work possible.
5. R. B. Bernstein, J. Phys. Chem. 56, 893 (1952).
6. ———, in *Proceedings of the International Symposium on Isotope Separation* (North-Holland, Amsterdam, Netherlands, in press).
7. G. A. Ropp, A. J. Weinberger, O. K. Neville, *J. Am. Chem. Soc.* 73, 5573 (1951).
8. P. E. Yankwich and R. Haschemeyer, in preparation.
9. I appreciate the help of D. E. Marsh and C. D. Miller with the mass spectrometer.

19 April 1957

Lung Volume of Amphibian Tadpoles

In 1949 Witschi (*1*) described, in tadpoles of the genus *Rana,* a pair of fibrous strands which connect the round windows of the otic capsules with the bronchi. In the following year (*2*) he noted that larvae of *Xenopus* possess small diverticula of the bronchi which also come into contact with the round windows of the ears. Witschi called these structures bronchial columellae and bronchial diverticula, respectively.

It occurred to Witschi (*1, 3*) that these connections of the gas-filled lungs with the ears might serve for the transmission of pressure changes, particularly sound vibrations. In order to make quantitative statements about sound and pressure reception by the lung, the lung volume is an important quantity to be known.

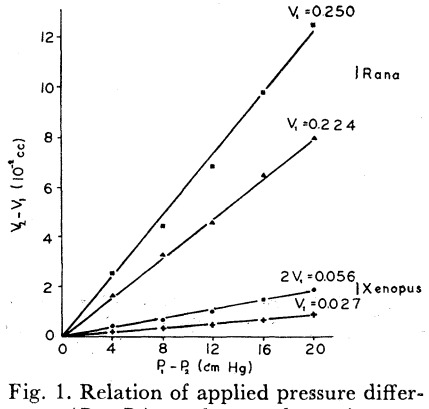

Fig. 1. Relation of applied pressure difference $(P_1 - P_2)$ to lung volume increase $(V_2 - V_1)$ in larvae of *Rana* and *Xenopus*. The calculated original lung volume is indicated in cubic centimeters at each graph. Note that one curve for *Xenopus* represents two animals $(2V_1 = 0.056\ cm^3)$.

Since dissection of the lungs and subsequent determination of their volume introduces uncontrollable errors because of manipulation, it was decided to devise a technique for measurement in the living animal.

Let a specimen of *Rana* species be swimming free in a vessel under atmospheric pressure P_1. The volume of the animal's lung may be denoted by V_1. Then, if the vessel is partially evacuated to pressure P_2, the volume of the lung (assuming passive behavior of this organ) will increase to V_2, such that

$$V_1P_1 = V_2P_2$$

(temperature T constant). By suitable manipulation, this expression may be restated

$$V_1 = \left(\frac{P_1}{P_1 - P_2} - 1\right)(V_2 - V_1)$$

Since P_1 and P_2 are known, it is necessary to know only $(V_2 - V_1)$, or the increase in volume. To measure this quantity, one or more animals were enclosed in a small vessel completely filled with water. Through the stopper of this vessel, a calibrated capillary protruded, which was approximately half filled with water. Thus, the only free air present in the system is the air in the lungs of the animals. This small vessel was put in a larger vessel, in which a volume of water served as a temperature buffer, so that no changes in temperature would occur during an experiment; a thermometer was added to check this. The large vessel was then evacuated to a preset pressure in the range of 56 to 76 cm-Hg, and the rise in level of the fluid in the capillary was measured. Since the capillary was calibrated, the rise in level could be read directly as 10^{-2} ml increase in volume.

It was assumed that the lung expanded passively; if this assumption is correct, the same value for V_1 must obtain from any set of P_1 and P_2, or in a graphical representation, the relation $(P_1 - P_2)$ versus $(V_2 - V_1)$ should be a straight line. For each animal or set of animals, such a straight line was obtained. Figure 1 shows four cases, two of *Rana catesbeiana* and two of *Xenopus laevis*. It is apparent that the slope of a particular relation is determined by the initial volume, V_1, which is indicated in the graph. In a series of measurements on single *Rana catesbeiana larvae* of stages 28 to 29 (Witschi, *4*) values of V_1 ranged from 0.14 to 0.42 cm^3, with the mean at 0.28 cm^3. *Xenopus laevis* larvae of similar age were measured in groups of three, four, and ten individuals, and the total lung volume was divided by the number of animals. Thus, values of V_1 ranged from 0.020 to 0.034 cm^3 with the mean at 0.026 cm^3. Since *Rana* larvae are heavier than water and tend to sink to the bottom, while *Xenopus* larvae are lighter and have to make continuous effort to keep from floating up (*5*), one is led to infer that *Xenopus* larvae carry relatively more air than *Rana* tadpoles. It is then meaningful to express the lung volume as percentage of body volume. Therefore, the body volume of the animals was measured before each pressure experiment by dropping them in an appropriate graduated cylinder and noting the rise of the meniscus. Thus it appears that *Rana* larvae have an average lung volume amounting to 2.3 percent of body volume; *Xenopus* larvae, on the other hand, maintain an average of 3.7 percent air.

From these results, it is possible to obtain an upper and lower bound of the specific gravity of the tadpole as a whole. Assuming a floating condition (specific gravity, 1.0000), where the air just cancels the weight of the tissue in excess of the weight of an equal volume of water, one may calculate from the quoted percentages that the specific gravity lies between 1.020 and 1.034.

A measurement was carried out to determine the specific gravity of *Xenopus* larvae in the following manner. A small number of larvae were homogenized in a known volume of water. The total volume was determined after homogenization, which thus yielded the volume of tadpole tissue. From this 1-cm^3 samples were weighed; after correction for the added water, these weighings averaged at 1.025 for the specific gravity of *Xenopus* larval tissue (*6*).

WILLEM A. VAN BERGEIJK*
Zoology Department, State
University of Iowa, Iowa City

References and Notes

1. E. Witschi, *Z. Naturforsch.* 46, 230, (1949).
2. ———, *Anat. Record* 108, 590 (1950).
3. ———, *J. Morphol.* 96, 497 (1955).
4. ———, *Development of Vertebrates* (Saunders, Philadelphia, Pa., 1956).
5. W. A. van Bergeijk, *Anat. Record* 120, 754 (1955).
6. This work was aided by a contract between the State University of Iowa and the Office of Naval Research, Department of the Navy (NR 163-145).
* Present address: Bell Telephone Laboratories, Inc., Murray Hill, N.J.

15 April 1957

Leaching of Carbohydrates from Plant Foliage as Related to Light Intensity

Modern use of radioactive isotopes has effectively demonstrated the loss of mineral nutrients from plant foliage by leaching with aqueous solutions (*1, 2*). In addition, large amounts of organic materials, principally carbohydrates, can

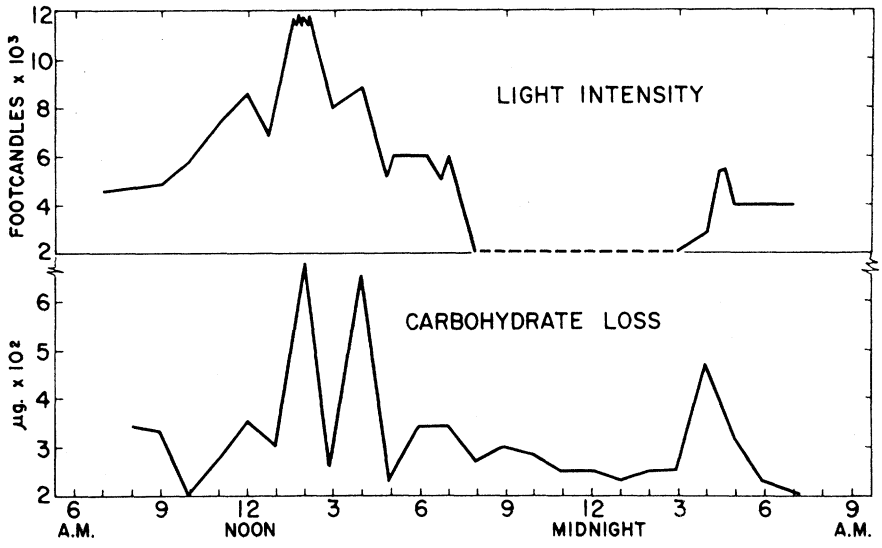

Fig. 1. Loss of carbohydrates from bean leaves by leaching with distilled water as related to light intensity. (Top) Hourly variation of light intensity in foot candles; (bottom) average hourly carbohydrate loss in micrograms per leaf.

be leached from leaves (*1, 3*). During the course of leaching studies at our laboratory, an interesting relationship between carbohydrate loss and light intensity has been noted and is herein reported (*4*).

Bean seeds (*Phaseolus vulgaris,* var. Contender) were germinated in sand, and the seedlings were transferred to aerated cultures containing half the nutrient intensity of Hoagland's standard solution (*5*). After 2 weeks, an entire primary leaf, while still attached to the intact plant, was immersed in 130 ml of distilled water in a flat vessel. Leaves on six plants were used for replication. At hourly intervals for 24 consecutive hours, including the natural daylight and dark periods, the solutions in the vessels were drawn off and replaced with fresh distilled water. The solutions containing the leaf leachate were evaporated on a steam bath to a standard volume of 10 ml and analyzed colorimetrically for carbohydrates (*6*). The plants were harvested at the end of the leaching period; the leaves which had been leached were removed, and dry weights were determined. Light intensities were recorded during the experimental period.

The results of the study are presented in Fig. 1. The upper half of the graph shows hourly variations of light intensity in foot candles, and the lower half shows the hourly losses of carbohydrates in micrograms per leaf. The two curves are remarkably similar, showing an apparent direct relationship between light intensity and the leaching of carbohydrates. It will be observed that greatest removal of carbohydrates occurred during the periods of highest light intensity. Only small losses occurred during darkness. The total loss of carbohydrates during the 24-hour period was 7.5 mg per leaf, which was 4.8 percent of the dry-weight equivalent of the leached leaves. Variation among the replications was slight in both total and hourly losses. The principal carbohydrate leached from bean leaves under similar conditions has been identified as a galactan (*1*).

To substantiate further the relationship between carbohydrate loss and light intensity, two variations were introduced into the afore-described experimental procedure: (i) the leaf being leached was left exposed to light, and the remainder of the plant was covered with a black cloth; and (ii) the leaf being leached was covered with a black cloth, and the remainder of the plant was exposed to light.

In general, the results were similar to those reported earlier in this paper. When the leaf was left exposed to light (variation i), loss of carbohydrates paralled the intensity of light; and when the leaf was covered with the cloth (variation ii), carbohydrate losses were constantly low and did not fluctuate with the changes of light intensity. Further, although the temperature of the leaching solution and the covered leaf (variation ii) rose somewhat during the hourly intervals, no relationship between these rises and the carbohydrate loss could be determined. This tends to show that the light intensity, and not the temperature increase, was the factor associated with carbohydrate loss from the leaves.

Two hypotheses may be suggested to explain these phenomena. First, increased solar radiation stimulates the photosynthetic activity of the leaf. Since newly elaborated carbohydrates are readily water soluble, they are in a condition to be easily leached immediately after manufacture. Second, the mechanism of carbohydrate removal may be affected. There is evidence that the number of plasmodesmal connections from the cells to the leaf surface increases in the light (*7*). The plasmodesmata may aid in establishing a pathway for carbohydrates to be leached from the leaf.

Varying amounts and intensities of rainfall occur in different parts of the world and at different seasons of the year. In addition, many modern agricultural practices—for example, overhead irrigation, mist propagation, syringing, and spraying—involve the application of aqueous solutions to aboveground parts of plants. It has been shown that more than 400 kg of carbohydrates per acre can be removed by rain from the foliage of apple trees during a single growing season (*3*). The close relationship between light intensity and carbohydrate losses by leaching and the significance of these losses, especially at critical times in plant development, await further evaluation.

H. B. Tukey, Jr.
S. H. Wittwer
H. B. Tukey
Department of Horticulture, Michigan State University, East Lansing

References and Notes

1. W. G. Long, D. V. Sweet, H. B. Tukey, *Science* 123, 1039 (1956).
2. M. G. Mes, *S. African J. Sci.* 50, 167 (1954); H. B. Tukey, Jr., S. H. Wittwer, H. B. Tukey, *Abstr. Am. Soc. Hort. Sci. Ann. Meeting,* Storrs, Conn. (1956).
3. S. Dalbro, *Proc. 14th Intern. Hort. Congr.,* in press.
4. This investigation was supported by contract No. AT-(11-1)-159 of the U.S. Atomic Energy Commission, Biological and Medical Division. This is journal article No. 2034 of the Michigan State Agricultural Experiment Station.
5. D. R. Hoagland and D. I. Arnon, *Calif. Agr. Exp. Sta. Circ.* 347 (1950).
6. E. Lunt and D. Sutcliffe, *Biochem. J.* (*London*) 50, 122 (1953).
7. P. Lambertz, *Planta* 44, 147 (1954).

6 May 1957

Thyroxine Effect on Melanophore Contraction in Xenopus laevis

While working with lathyrism in the South African toad, *Xenopus laevis* Daudin, we observed that tadpoles became blanched when they were treated with thyroxine solution. A series of experiments was then designed to investigate the role of thyroxine in the color control mechanism of this species (*1*).

It is well known that in both adult and larval *Xenopus,* removal of eyes results in expansion of the epidermal melanophores (*2*). Figure 1*a* illustrates a skin preparation from a newly blinded tadpole showing the expanded melanophores. When such animals are placed in L-thyroxine-Na solution of various con-