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13. Glutamate and aspartate determinations performed by the enzymatic method of L. T. Graham and M. H. Aprison [*Anal. Biochem.* **15**, 487 (1966)] yielded no evidence of an increase in the concentrations of these substances in perilymph collected during acoustic stimulation as compared with perilymph collected in silence. If ANAS is, or contains, the primary afferent transmitter, that transmitter cannot be glutamate or aspartate.
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16. 115 m*M* NaCl, 1.1 m*M* KCl, 1.2 m*M* $CaCl_2$, 5.5 m*M* glucose, and 2.7 m*M* $NaHCO_3$.
17. The sound stimulus consisted of bursts of white noise of 90 msec duration at ten bursts per second presented in free-field configuration. The white noise intensity measured with the use of octave band filters ranged between 57 dB sound pressure level (SPL) at 125 Hz and 73 dB SPL at 2 kHz. On the C scale of a Bruel and Kjaer sound level meter, an intensity of 78 dB was registered.
18. The sound stimulus consisted of continuous white noise plus clicks presented at seven to ten per second in free-field configuration. The white noise intensity ranged between 45 dB SPL at 125 Hz and 85 dB SPL at 4 kHz. An intensity of 88 dB SPL was registered. The click intensity ranged between 44 dB at 31.5 kHz and 86 dB at 4 kHz. An intensity of 92 dB SPL was registered. This combination of white noise plus click was presented for 5 to 7 minutes with a 5-minute rest period between presentations.
19. C. H. Norris and P. S. Guth, *Acta Otolaryngol.* **77**, 318 (1974). Perilymph was collected by means of a single-barrel micropipette placed in the scala tympani of the basal turn rather than by perfusing the perilymphatic space with artificial perilymph.
20. During the 2-year period of this research, neither guinea perilymph nor bullfrog perfusate, collected during acoustic stimulation, caused an increase in firing rate during the winter months (October through March). This experience recalls the early statement of R. M. Yerkes [*J. Comp. Neurol. Psychol.* **15**, 279 (1905)]: "evidently the seasonal condition of the animal is an important matter to consider in studies of audition."
21. Supported by Veterans Administration grant VA 71-6 (to P.S.G.), Public Health Service grant NS-11647 to the Kresge Hearing Research Laboratory of the South, a John A. Hartford Foundation grant (to C.H.N.), and Public Health Service training grant IT32 G2107172-02 (to W.F.S.). We thank R. R. Capranica for advice and help.

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Use of Indigenous Rubidium to Trace Potassium Fertilizer in the *Pinus resinosa* Ecosystem

The conclusion by Stone and Kszystyniak (*1*) that K was being conserved in the *Pinus resinosa* ecosystem was based upon reduced Rb/K ratios in plants and soil after application of K fertilizer low in Rb. We question the implicit assumption, critical to their conclusion, that the absolute amount of Rb in the soil was not reduced by K fertilization.

In the "reverse tracer" technique of Hafez and Stout (*2*), which Stone and Kszystyniak used, it is assumed that K and Rb remain independent of each other in the soil. However, addition of K may fundamentally change the movement of Rb through the soil-plant system. Fertilization with K may reduce the absolute amount of Rb taken up from the soil by plants as a result of competitive uptake (*3*). This action could also increase the loss of Rb from the ecosystem by leaching, because the sink for Rb in the biomass is reduced by this process. Therefore, the assumption that Rb remains unaffected by K fertilization may not be justified.

Data presented in (*1*) are inadequate to permit one to distinguish between retention of K by ecosystem processes and loss of Rb by leaching. Reduced Rb/K ratios in plant tissues could result from either K retention or Rb loss. The analyses of litter (in milligrams per kilogram of litter) showed reduced concentration of Rb (*1*). In soil (0 to 15 cm deep), the Rb concentration was reduced relative to that of the controls in one stand (Russia, New York) but was essentially constant in the other (North Lawrence, New York) (*1*). Thus soil data showed a possible reduction in Rb concentration in one of the two cases presented. From these data, the loss of Rb after K fertilization cannot be ruled out as a contributing factor in the reduced Rb/K ratios observed.

The reverse tracer technique is potentially useful for investigating the retention of K by ecosystems. However, for valid conclusions to be drawn, the movement of both K and Rb through all parts of the ecosystem, and particularly the interactions between these elements in the soil, must be considered.

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Ryan and Miller raise the possibility that added K may cause leaching of native soil Rb beyond the effective depth of root uptake (*1*) and so account for part of the reduction in plant Rb/K that we reported (*2*). They cite in support the reduced content of 1*N* HNO_3-extractable Rb in the upper 15-cm layer of soil at our Russia, New York, site 23 years after surface application of 112 kg of K per hectare.

There are several objections to this reasoning. (i) Even if the added K had been wholly retained within the upper 15 cm, it would have been equivalent to only about 5 percent of the total exchange capacity, as determined at ambient *p*H. Hence the opportunity for simple mass action displacement of even exchangeable Rb was slight. (ii) Studies of the Rb/K system in mineral soils demonstrate preferential absorption or retention of Rb in both exchange and fixation reactions (*3*). (iii) The samples cited had been subject to the cumulative influence of 23 years of returned litter with a reduced Rb/K ratio, and of canopy wash with presumably a similarly reduced ratio. Studies in a quite different forest show that the exchangeable Ca/Mg ratio of the surface soil approached that of the returned litter despite large point-to-point variations in Ca content (*4*).

The most direct evidence, however, comes from experimental results from a location adjacent to our Russia site. Gritzinger (*5*) measured 1*N* HNO_3-extractable Rb in successive depths of soil 4 months after surface application of 216 kg of K per hectare as K_2SO_4. Mean extractable Rb contents (in milligrams per kilogram of soil for control and fertilized plots, respectively), were as follows: 0 to 10 cm, 0.86 and 0.82; 10 to 20 cm, 0.65 and 0.70; 20 to 30 cm, 0.83 and 0.92; 30 to 40 cm, 0.98 and 1.13; and 40 to 50 cm, 1.11 and 0.93. None of the differences within depth are significant at the 5 percent level. Yet the total precipitation over this period (July to October) was 708 mm, providing opportunity for leaching. During the same period the K concentrations of pine foliage, stems, and roots from treated plots increased and their Rb/K ratios decreased, demonstrating the uptake of applied K. The absence of appreciable displacement of native Rb, even in the surface layer where the added K was initially concentrated, further indicates that the concern expressed by Ryan and Miller is unfounded.

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