chamber, and excellent agreement was reached.

All the films were sent for development and measurement to the New York Health and Safety Laboratory of the U.S. Atomic Energy Commission. Accompanying each exposed film was an unopened film with the same history of humidity and temperature. The films were compared on a densitometer. (*3*).

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3. We thank the personnel of the New York Health and Safety Laboratory of the U.S. Atomic Energy Commission for the loan of the crystals and the measurement of film densities. Our work was supported by grants from the Nuclear Atomic Energy Commission of Brazil.

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Evaluation of Isotope Exchange by Chromatography

Abstract. The study of exchange reaction kinetics between sulfur-35 and organic compounds which contain sulfur can be made much easier by use of radiochromatography. Activity ratios of the components of the reaction mixture can be estimated by use of very small amounts of radioactive materials. The reaction need not be halted and individual components need not be isolated. Any degradation of reacting substances can be measured quantitatively at the same time.

Much attention has been devoted recently to the development of methods for labeling biological materials with radioactive isotopes. In addition to chemical synthesis and to a much lesser extent biosynthesis, extensive use has been made of exchange reactions between inactive organic substances and simple inorganic active isotopes (*1*). Properly employed, this method permits easy labeling of highly complicated molecules, even those that are difficult to synthesize (*2, 3*).

In order to obtain the best results from exchange reactions, it is necessary to know rather precisely the most favorable experimental conditions. Exchange reactions are controlled by temperature, time, concentration of components, catalysis, and the type of reaction medium. The rate of degradation of the substance being labeled also has an effect. A series of time-consuming assays must be carried out to measure the effects of these factors on the exchange reactions. The step-by-step exchange reactions of radioactive sulfur have been estimated on the basis of the increase in specific activity of the organic substance or by measuring the decrease in specific activity of radioactive sulfur. The exchange reaction must be halted, however, to make these assays (*4*).

In our studies of the most efficient use of elemental S^{35} in exchange reactions (*2*), we have used radiochromatography as a rapid means for following and evaluating the course of exchange and the rate of degradation of the substances being examined. The technique is easier and simpler than the assay procedure.

The technique provides a rapid means for following step-by-step exchange without having to stop the reaction. In addition, it substantially reduces the number of experiments and also the amount of radioactive material needed. If a suitable chromatographic system is used, only a few milligrams of the substance to be labeled and only about 1 μc of S^{35} are needed to study the exchange and any degradation that may occur.

At specified time intervals, small samples are taken out of the homogeneous reaction mixture with a capillary tube. The samples are spotted directly on a sheet of chromatograph paper. The paper is then developed by means of a system which separates the source of radioisotopes from both the substance being labeled and from any degradation products. In most systems—for example, in water-saturated butanol—elemental sulfur has rather high R_F values. The developed chromatograms can be used with adequate accuracy and reproducibility (*5, 6*) for estimating activity ratios of individual components at the instant of sampling. Our method deals with the estimation of radioactivity *ratios* only. The measuring instrument need not be calibrated with standard samples. Chromatograms are resolved by planimetric determination of the areas outlined by graphic recording of activity distribution. Radioactivity is measured by means of an automatic device (Frieseke-Hoepfner) for continuous scanning of radioactive chromatograms.

By determining activity ratios of exchange reaction components when the system is at equilibrium one can determine the exchange equilibrium constant, the exchange coefficient α, and the percentage exchange. For example, the simple exchange between elemental S^{35} and an organic compound can be expressed by the equation

$$SS^* + OS \rightleftharpoons SS + OS^*$$

where SS^* is the radiosulfur molecule (the asterisk denotes a radionuclide) and OS is an organic compound. The equilibrium constant K of the exchange is

$$K = \frac{[SS]\ [OS^*]}{[SS^*]\ [OS]}$$

and the exchange coefficient α is

$$\alpha = \frac{[S^*]}{[S]_{OS}} : \frac{[S^*]}{[S]_{SS}}$$

In this case the numerical value of K equals α and it can be expressed by the relationship

$$K = \alpha = \frac{a}{b}\left(\frac{x_0 - x}{x}\right)$$

where a is the total concentration of the compound in the reacting mixture $[OS^*] + [OS]$, b is the total concentration of the organic compound in the reacting mixture, x_0 is the ratio $[SS^*]/[SS^* + SS]$ at the start of exchange, and x is the same ratio, but at a point when equilibrium is reached (*7*).

At the early stage of reaction the concentration SS equals zero (no radioactive sulfur has yet entered from the reacting mixture into the organic compound) and $x_0 = 1$. To compute K or α it is necessary only to determine from the chromatograms the value of x, because

$$\alpha = \frac{a}{b}\left(\frac{1 - x}{x}\right)$$

The value of x denotes the unreacted portion of S^{35} at the equilibrium point. This value can be readily obtained from the recorder diagram as the quotient of the radioactivity value of the area belonging to S^{35} divided by the sum of the radioactivity of this area and the radioactivity of the area belonging to the organic compound. The radioactivity of the organic compound comes exclusively from the exchange reaction with elemental S^{35}.

Radiochromatographic evaluation of exchange reactions or of components of a reacting mixture can also be applied to carbon-14 and even to tritium (*6*) if the physical properties of the reacting substances are appropriate.

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Use of Iodine-131 To Measure Movements of Small Animals

Abstract. A technique utilizing iodine-131 was developed to study the movements of *Microtus pennsylvanicus* in the field. Iodine-131 (½ mc) was placed in a polyethylene capsule and inserted beneath the animal's skin. Movements of the animal were then followed for periods of up to 2 weeks by means of a Geiger-Müller counter attached to an aluminum pole.

Radioactive isotopes have been used for approximately 12 years to measure movements of small mammals in the field [Brian (*1*) had used a radioactive isotope (radium sulfate) to study the movements of click beetles as early as 1947.] Godfrey (*2*) placed cobalt-60 in metal leg bands to study the movements of *Microtus agrestis* and Kaye (*3*, *4*) used gold-198 wire, injected subcutaneously, to study the movements of *Reithrodontomys humulis humulis*. Thus, it may be seen that the use of radioactive isotopes to trace the movements of small mammals is not a new technique. However, it was felt that certain modifications might make the isotope technique a much more useful research tool to the field biologist. The method reported here was developed and used to study movements of *Microtus pennsylvanicus* within a bluegrass-timothy seral community.

Iodine-131 dissolved in a sodium sulfite solution was selected for use in this method for the following reasons: (i) the isotope in this form can be handled easily and with minimum contamination of equipment; (ii) its half-life of 8.1 days and the fact that it could be used by other investigators at this university made its use quite inexpensive; (iii) the 8.1-day half-life also makes I^{131} safer to use in the field than an isotope such as Co^{60}; and (iv) I^{131} is a high-energy gamma emitter (0.08 to 0.72 mev), which makes detection in the field relatively easy. To prevent rapid absorption and excretion of the isotope by the animal, the I^{131} was placed in a polyethylene capsule.

The polyethylene capsule was constructed from polyethylene tubing of two sizes, with outside diameters of 0.043 and 0.062 inch, respectively. A 1-inch piece of the smaller tubing was inserted, to a depth of ½ inch, into each end of a 2-inch piece of the larger tubing, which later served as the main body of the capsule. Occasionally it was necessary to seal the joints between the smaller and larger tubing with a drop of Duco cement to assure an airtight seal. A hypodermic needle with a syringe attached was inserted into one end of the unfinished capsule, and a small amount of sodium sulfite solution containing I^{131} was then drawn into the tubing. The amount of activity in the capsule can readily be adapted to experimental conditions by changing the volume or concentration of the I^{131} solution drawn into the tubing. In our field work 0.5 mc of I^{131} was placed in each 1-inch capsule. When the proper amount of solution had been drawn into the tubing, the end of the tubing was removed from the stock solution. The solution in the tubing was then drawn into the central portion. The two ends of the tubing were then cut and sealed with an ordinary woodburning tool.

The reason for placing a section of the smaller tubing in each end of the larger tubing was twofold: (i) it provided additional polyethylene to assure a good seal when the overlapping ends of the tubing were cut and sealed with the woodburning tool, and (ii) it made it possible to place I^{131} solution in the tubing without contaminating the external surface of that part of the tubing which formed the capsule.

After the capsule had been prepared it was inserted subcutaneously into a mouse by the following method. The capsule was placed in a 12-gauge hypodermic needle. The needle and capsule were then inserted beneath the skin of the mouse. The capsule was implanted beneath the animal's skin by gradually withdrawing the needle while pushing the capsule out by means of a wire rod. This insertion technique had been previously used by Kaye (*3*) to insert Au^{198} wire beneath the skin of *Reithrodontomys humulis humulis*. Usually four animals were tagged and released during each study period. Each of these animals had been trapped at least four or five times prior to tagging. This precaution was taken in an attempt to prevent the tagging of transient animals and to gain some idea of the approximate area within which the animal might be found after it had been tagged.

After its release at the point of capture the mouse was followed by means of a Thyac model 389C survey meter attached to an 8-foot aluminum pole (Fig. 1). A Geiger-Müller beta window tube with a wall thickness of 30 mg/cm^2 was placed in the end of the pole, and a small window was cut in the pole beneath this Geiger-Müller tube to facilitate detection of the gamma radiation from the I^{131}. The operator made

Fig. 1. The instrument used to track I^{131}-tagged *Microtus pennsylvanicus* in the field.