ally, the data obtained with O^{18} are consistent with an ionic mechanism (—COO^- R^+) and suggest the possibility that PAH transport may involve an ion-exchange mechanism such as has been postulated in the tubular excretion of hydrogen (*12*) and potassium ions (*13*).

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19 June 1956

Method of Preparing Radioactive Cations for Tracing Ground Water

In recent years there has been considerable interest in the use of radioisotopes as ground-water tracers. The properties and use of several types of chemical and radioactive tracers have been described (*1*).

Because of adsorption in the soil by cation exchange, the use of radioactive tracers has been limited almost entirely to the two anions iodine (I^{131}) and bromine (Br^{82}); the other radioactive anions are unsuitable for other reasons. The fact that there are only two suitable radionuclides limits the experimenter in his choice of half-life, type of radiation, and energy. It is a further unfortunate circumstance that iodine has one of the lowest permissible concentrations in water (4.8×10^{-4} $\mu c/cm^3$ for an 8-hour period) of any of the radioisotopes (*2*); radioactive bromine presumably has a similarly low permissible concentration.

However, it is possible to reduce greatly the adsorption by the soil of certain of the normally cationic radioisotopes by chelation—that is, by binding them into a un-ionic form. This has been done with Versene (*3*), the tetrasodium salt of ethylenediamine tetraacetic acid. With this simple procedure, consisting of adding Versene to a solution of the radioisotope, it is possible to use a number of radioisotopes with widely different half-life and radiation energy as ground-water tracers. Virtually all of these isotopes are much less hazardous than I^{131} or Br^{82}.

Laboratory tests using Co^{60}, Sb^{124}, and Cr^{51} have been made. Versene is nonspecific in its action on metal ions; it inactivates the normal ionic properties of almost all polyvalent cations by forming with them water-soluble chelates that are extremely stable, except when they enter a solution containing metallic ions capable of forming more strongly chelated compounds. Such ions would displace the metals from the more weakly chelated compounds, exposing them to the possibility of adsorption on particles of earth material. Therefore, metals chosen for use as tracers should be capable of forming more strongly chelated compounds than can the common cations in the ground water, of which calcium is the strongest. In practice this means that they should be above calcium on the "chelation scale" at *p*H values characteristic of natural water—about 7. The order of chelation of some of the common metals at *p*H 8.65 is as follows:

$$Ni > Co > Cu > Zn \text{ or } Cd > Ca > Mg > Sr > Ba.$$

Processed Co^{60} of high specific activity (5.3-year half-life), Sb^{124} (60-day half-life), and Cr^{51} (26.5-day half-life) were used as the radioactive tracers. They were obtained as chlorides in less than 1*N* HCl. Five microcuries of each tracer was divided into two equal portions. To one portion, about 10 mg of reagent-grade Versene was added to chelate the radiocationic tracer, and then each portion was diluted to 250 ml using Oak Ridge tap water.

The solution containing the tracer was allowed to percolate slowly (4 ml/min) through an 18-mm column containing 10 g of a fine-grained, somewhat weathered calcareous shale, which has an ion-exchange capacity, as determined by using ammonium acetate, of about 30 milliequivalent/100 g.

Samples of the chelated and unchelated influents and effluents were prepared for nuclear counting. The relative concentrations of radioactivity in the effluents from the column (Fig. 1) show the extent to which chelation inhibited

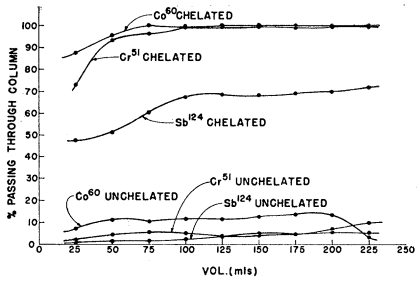

Fig. 1. Test results showing percentage adsorption of chelated and unchelated radioisotopes.

the adsorption of the radioisotopes by the shale. The shale used is believed typical of earth materials having a relatively high adsorption capacity for radiocations (*4*), but the scale of the experiment makes it unwise to predict that all chelated cations will be sufficiently free from adsorption to be satisfactory under all conditions. However, it is hoped that more laboratory work and some field tests can be reported later.

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Inhibition of Yeast Hexokinase by Fluoride Ion

It has been repeatedly demonstrated that various hexokinases require magnesium ion for optimal activity (*1, 2*). It has also been reported that magnesium-activated yeast hexokinase is not affected by fluoride ion in concentrations exceeding 0.1*M* (*1*), and fluoride is, in fact, often added to tissue extracts to inhibit adenosine triphosphatase (ATPase) during measurements of hexokinase activity (*3*). Since the solubility of MgF_2 is very

low (*4*), there is an apparent conflict between the requirement of the enzyme for magnesium and the lack of effect of concentrations of fluoride which are incompatible with the maintenance of appreciable levels of magnesium ion. In this communication it is shown that the order of mixing is of the utmost importance in determining the effect of fluoride in this system. These results place definite limits on the use of fluoride in assays for hexokinase activity, and their implications require consideration in every case in which fluoride is used with ATP-requiring enzyme systems.

The assay system utilized the fact that hydrogen ion is produced as the hexokinase reaction proceeds (*1*). In each case, cresol red (*5*), glucose, hexokinase, magnesium chloride, and sodium fluoride (replaced by chloride in control samples) were mixed and preincubated for the specified times. The reaction was started by the addition of a solution of ATP in triethanolamine buffer, and the absorption at 5710 A was measured at intervals using a Beckman spectrophotometer. Final concentrations were 1m*M* glucose, 1m*M* ATP, 1m*M* Mg^{++}, 0.1*M* F^- (or Cl^-), 2m*M* triethanolamine (*p*H 8.4), 4μg/ml of cresol red, and 10μg/ml of yeast hexokinase (Sigma "practical") in a total volume of 3 ml. Temperature was controlled at 25°C. It is to be noted that under these conditions the enzyme is saturated with glucose and is sensitive to (Mg^{++}).

Results of a typical experiment are shown in Fig. 1. When the mixture contained fluoride, the time of preincubation markedly affected the measured rate of the reaction. However, the degree of inhibition did not increase during the measurement. This is emphasized in Fig. 1 by plotting the negative logarithm of the concentration of ATP against time, which shows the close adherence to first-order kinetics.

It was further observed that, although the relatively small inhibition obtained after 1 minute of pre-incubation with fluoride and the almost complete loss of activity observed after a 30-minute exposure were quite reproducible, the degree of inhibition obtained after 15-minute preincubation periods was extremely erratic. The following values of the rate constant were obtained in a series of replicate determinations: 1 minute of preincubation with fluoride, 0.032, 0.032, and 0.034; 15 minutes, 0.030, 0.023, 0.0023, 0.034, and 0.0069; 30 minutes: 0.0046 and 0.0051. In other experiments it was found that addition of sodium fluoride 1 minute *after* the other components had been mixed had no measurable effect on the rate of the reaction. This confirms previous reports (*1*).

All these observations can be explained on the basis of the assumption that the rate of removal of magnesium ion from solution by fluoride ion is a relatively slow process. If it is assumed that this occurs as follows:

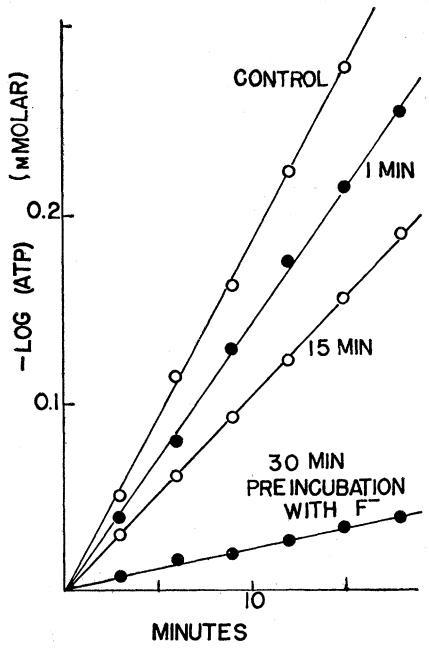

Fig. 1. Inhibition of yeast hexokinase by fluoride. The reaction was started by addition of ATP after preincubation of the other constituents for the times indicated on the curves. Chloride was substituted for fluoride in the control. Final concentrations are listed in the text.

$$Mg^{++} + F^- \rightleftarrows MgF^+ \quad (1)$$

$$MgF^+ + F^- \rightleftarrows MgF_{2(aqueous)} \quad (2)$$

$$MgF_{2(aqueous)} \rightleftarrows MgF_{2(solid)} \quad (3)$$

it seems probable that reaction 3 represents the rate-determining step in the removal of magnesium ion. The separation of a solid phase is known to depend on many factors and is frequently slow. If reactions 1 and 2 are more rapid than reaction 3, the rate of precipitation of MgF_2 would depend, among other things, on the concentration of magnesium ion. The addition of ATP to such a mixture would affect this process by reducing the concentration of free magnesium ion because of the formation of a magnesium-ATP complex as follows:

$$Mg^{++} + ATP^{-4} \rightleftarrows MgATP^{--} \quad (4)$$

This reaction occurs rapidly, and since the formation constant is of the order of 2×10^4 (*6*), the concentration of free magnesium ion in the systems studied is markedly reduced by the addition of ATP. Consequently the rate of reaction 3 is reduced to such an extent that no further effect of fluoride is detected over the period of observation.

This formulation accounts for the fact that no inhibition is observed when ATP is mixed with magnesium ion prior to the addition of fluoride, as well as the observations that fluoride mixed with magnesium ion prior to the addition of ATP exerts an inhibitory effect that is related to the time of preincubation, but does not increase after ATP is added.

The scatter in the measurements made after 15 minutes of pretreatment with fluoride is probably due to variations in the rate of reaction 3, inasmuch as the separation of a solid depends on the chance of finding or forming centers suitable for nucleation, and wide fluctuations in this variable are to be expected when mixtures of this type are made. The relatively constant inhibition found after 1 minute of preincubation may correspond to the extent of reactions 1 and 2 alone, while the considerable inhibition found after 30 minutes probably reflects a close approach to equilibrium precipitation of magnesium fluoride.

We conclude that fluoride ion can inhibit yeast hexokinase by removal of magnesium ion. This inhibition requires a finite time, and it can be minimized by attention to the order of mixing of reagents. Since it is probable that the observed effects are due to variations in the rate of precipitation of magnesium fluoride, we suggest that this variable should be evaluated in the interpretation of any enzyme assay in which fluoride is used (*7*).

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Autoinhibition of Bacterial Endospore Germination

In the last few years, a considerable number of published works on the formation and germination of bacterial endospores has appeared. Many of these studies have been concerned with the physiological aspects of the processes. Details of the more significant investigations on this subject have been recently