Reports

Renal Transport of *p*-Aminohippurate Labeled with Oxygen-18

In common with other active transport systems, the renal tubular excretion of *p*-aminohippurate (PAH) is presumed to involve an interaction between the transported compound and a cellular element (carrier), the nature of which is as yet unknown. The observation (*1, 2*) that this excretory pathway is shared by various carboxylic acids (PAH, 3,5-diiodo-4-pyridone-N-acetate, penicillin G, mentholglucuronide, and related compounds) and by sulfuric acid derivatives (phenolsulfonphthalein dyes, ethereal sulfates) suggests that the anionic group ($-COO^-$, $-SO_3^-$) is the primary site of attachment between the transported compound and carrier.

Table 1 summarizes clearance studies with PAH and certain derivatives in which potentially reactive groups have been chemically substituted. Introduction of a methyl group into the amide linkage (*p*-aminobenzoylsarcosine) or acetylation of the *p*-amino group yields products which are also actively excreted. In contrast, substitution in the carboxyl group (*p*-aminohippurhydroxamate) yields a product which, although bound to plasma protein only to the extent of 5 percent, is cleared by the kidney at a rate 14 percent below that of glomerular filtration. These findings support the view that the free carboxyl group is essential for the tubular excretion of PAH.

In an attempt to determine the nature of the chemical bonding between carboxyl group and carrier, PAH labeled with O^{18} has been employed (*3*). Information concerning the extent to which the O^{18} in the carboxyl group is retained or lost in the course of transport should set limits to the type of mechanism that may be involved. The PAH was prepared (*4*) from glycine containing 0.96 atom percent excess O^{18}; the recrystallized product contained 0.76 atom percent excess O^{18} in the carboxyl group. The labeled PAH was infused through a fine needle inserted directly into the exposed left renal artery of a lightly anesthetized dog, and urine was collected from the cannulated left ureter. The rate of infusion (0.7 mg/min) was sufficiently slow to ensure maximal extraction of PAH in a single passage through the kidney.

Over a period of 2 hours, 82 mg were infused into the artery and 72 mg appeared in the urine from the left ureter, giving a recovery of 88 percent of the PAH injected. At the termination of the arterial infusion, renal function studies were performed on the left kidney by standard clearance techniques. The glomerular filtration rate (creatinine clearance) was found to be 24 ml/min, and the effective renal plasma flow (PAH clearance) was 63 ml/min. With a filtration fraction (C_{CR}/C_{PAH}) of 0.38 and correction for the binding of PAH on plasma protein (filtrable fraction = 0.92) (*5*), it can be estimated that 35 percent of the PAH had been added to the urine by glomerular filtration and 65 percent by tubular excretion.

A portion of the original PAH and that recovered in the urine were converted to *p*-(*p'*-hydroxyphenyl)azohippuric acid by diazotization and coupling with phenol. After recrystallization from ethanol-water, each sample darkened at 244°C and melted with decomposition at 246°C (*6*); admixture with an authentic sample of *p*-(*p'*-hydroxyphenyl)azohippuric acid did not depress the melting point. The derivatives were pyrolyzed at 420°C for 60 minutes in the presence of $HgCl_2$, according to the method of Rittenberg and Ponticorvo (*7*).

Table 1. Studies performed on trained, unanesthetized, female dogs by standard clearance techniques. Comparison is made between the glomerular filtration rate (C_{CR}, creatinine clearance) and the clearance of the indicated compound (C_X) at plasma levels below 1 mg percent. A clearance ratio (C_X/C_{CR}) above 1 indicates tubular excretion. Trichloroacetic acid filtrates were used for the estimation of PAH and its derivatives (*2*).

Compound (X)	C_{CR} (ml/min)	C_X (ml/min)	C_X/C_{CR}
p-Aminohippurate	64	189	2.96
p-Aminobenzoylsarcosine	71	169	2.38
p-Acetylaminobenzoylsarcosine	64	139	2.18
p-Aminohippurhydroxamate	70	60	0.86

Under these conditions, the CO_2 liberated by pyrolysis is derived largely from the carboxyl and amide oxygens and only to a limited extent (about 5 percent) from the phenolic oxygen. The atoms percent excess O^{18} found in the CO_2 obtained from the control and experimental samples were 0.47 and 0.45, respectively. Correction for the dilution of carboxyl CO_2 by that derived from the unlabeled amide and phenolic oxygens ($3/2 \times 100/95$) yields figures of 0.75 and 0.72 for the respective samples. These values are in satisfactory agreement with the 0.76 atom percent excess O^{18} present in the carboxyl group of the original PAH. This experiment clearly demonstrates that the transport of PAH across the renal tubule did not result in any appreciable loss of O^{18} from the carboxyl group.

The study with O^{18}-labeled PAH serves primarily to exclude certain types of covalent bonding between the carboxyl group and carrier. Neither an amide (—CO—NHR) nor thiol ester (—CO—SR) linkage can be involved, since the formation of either entails the loss of carboxyl oxygen. An ester of the type (—CO—O—CH_2R) appears to be improbable, for, as Koshland (*8*) has pointed out, the acyl-oxygen bond (—CO—O—) is much more susceptible to hydrolytic cleavage than is the oxygen-methylene bond (—O—CH_2—).

The result obtained with labeled PAH is compatible with any of the following explanations. (i) The carboxyl group may not participate in transport in the manner presumed. The reasons for believing that it is an essential functional group have already been outlined. (ii) A carboxylic-phosphoric anhydride (—CO—O—PO_3HR) remains a possibility if neither its formation nor subsequent cleavage involves a loss of the original carboxyl oxygen. The dependence of PAH transport on ATP (*9*) may suggest the occurrence in transport of an adenylacylate, such as that described as an intermediate in acetate activation by Berg (*10*). Utilizing O^{18}-labeled acetate, Boyer *et al.* (*11*) have shown that the formation of acetyl-coenzyme A results in a transfer of carboxyl oxygen to adenylate. However, this finding may not be relevant to the present hypothetical situation, where cleavage of the anhydride could be effected by water and an appropriate acylase rather than by CoA and the acetate-activating enzyme. On the basis of the presently available information, it appears impossible to predict with certainty the relative labilities of the —CO—O— and —O—P— bonds to a specific enzymatic hydrolysis. (iii) Fin-

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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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

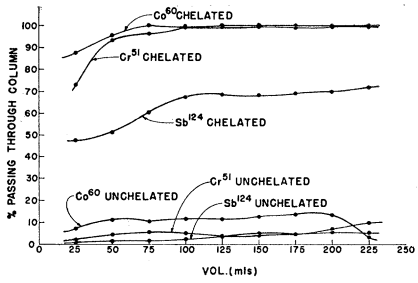

Fig. 1. Test results showing percentage adsorption of chelated and unchelated radioisotopes.

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