

Fig. 1. Starch-gel electrophoretic patterns of galactose-1-phosphate uridyltransferase from a normal subject and two homozygotes from the Duarte variant. The gel was prepared in 0.01M phosphate buffer, with 0.25M phosphate buffer in the buffer compartments. Each gel was photographed under ultraviolet light after application of the reaction mixture. The slots labeled N were filled with hemolyzate from cells of normal individuals. Those labeled D_1 and D_2 were filled with hemolyzate from the cells of the MO propositus and the BA proposita as described (2). Each preparation was diluted so that the total enzyme activity in each slot was the same, approximately equal to a 1:8 dilution of normal hemolyzate.

enzymes could be demonstrated either when the hemolyzates were diluted to the same enzyme activity (Fig. 1), or when diluted to the same protein concentration. An artificial mixture of normal and Duarte variant enzyme resulted in a broad band, the leading edge of which coincided with that of the Duarte variant and the trailing edge with that of the normal enzyme. Because of the relatively small quantity of mutant enzyme activity in heterozygotes, it is technically quite difficult to demonstrate clearly the presence of the variant in their hemolyzates. However, on careful examination such hemolyzates showed, in addition to normal enzyme, relatively faint fluorescence in a region just ahead of the normal band, corresponding to the Duarte variant.

To determine whether the difference in electrophoretic mobility between the two types of enzyme was due to differences in molecular size, electrophoresis was carried out not only in 16 percent, but also in 13 and 20 percent starch gel. It has been shown that retardation of larger molecules is greater when higher starch concentrations are used (5). The relative migration of the two enzymes was the same at all starch concentrations studied. The molecular weight of the two enzymes, partially purified on DEAE (diethylaminoethyl cellulose) (3), was also

estimated by gel filtration on Sephadex G-200. The material on the column was eluted with 0.15M sodium acetate solution containing 7 mM dithioerthyrotol and 0.27 mM EDTA (ethylenediamenetetraacetate). Alcohol dehydrogenase (molecular weight, 150,000, Boehringer) from yeast, serum albumin (molecular weight, 68,000, Calbiochem), horseradish peroxidase (molecular weight, 40,000, Sigma), and cytochrome c (molecular weight, 12,-000, Nutritional Biochemical Corporation), served as markers. Transferase in effluent from the column was estimated by a modification of the method of Maxwell et al. (3, 6). Under these circumstances 95 to 100 percent of enzyme was recovered. The elution pattern was highly reproducible; the molecular weight of normal enzyme was estimated in two filtration experiments at 87,000 and 83,500 and that of Duarte variant enzyme at 81,500 and 85,000.

Our studies indicate that individuals homozygous for the Duarte variant produce a structurally different galactose-1-phosphate uridyltransferase from that produced by normal individuals. Since the molecular weight of the normal enzyme and the Duarte variant enzyme appear to be approximately the same, the difference in migration in starch gel would appear to be due to differences in molecular charge or axial ratio. The charge difference appears much more likely, especially since changing the concentration of starch failed to influence the degree of separation of the two enzymes. Presumably the charge difference is due to an amino acid substitution. Since the kinetic properties of the enzyme do not appear to be abnormal, it is reasonable to suppose that the substitution does not affect the active site of the enzyme. but may well influence its rate of synthesis.

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Magnesium-28 in Rain: Produced by Cosmic Rays

Abstract. Existence of magnesium-28 (half-life, 21.3 hours) produced by cosmic rays in rain at concentrations of 1.7 and 6.1 \times 10⁻¹ atoms per milliliter was established radiochemically by isolating this nuclide from several hundred liters of rain samples collected at Fayetteville, Arkansas.

The following radionuclides, with indicated half-lives, produced by cosmic rays were known to occur in the atmosphere, or in atmospheric precipitation, or both, about 10 years ago (1): 2.7-million-year Be¹⁰, 5600-year C¹⁴, 12.5-year H³, 2.6-year Na²², 87-day S³⁵, 53-day Be⁷, 25-day P³³, 14-day P³², and 1-hour Cl³⁹. These radionuclides have since been added to the list of nuclides produced by cosmic rays: 700-year Si³² (2), 15.0-hour Na²⁴ (3), 37-minute Cl³⁸, and 2.8-hour S³⁸ (4).

Many nuclides with masses ranging from 25 to 31 are also expected to be produced by the cosmic-ray spallation reactions on atmospheric argon, but most of them are short-lived. Among these nuclides, 21.3-hour Mg²⁸ and 2.62-hour Si³¹ have half-lives long

enough to enable us to measure their concentrations in rain by the use of present-day, low-level counting and radiochemical techniques. In this work we have measured the concentrations of Mg²⁸ in rain by isolating this nuclide from large volumes of samples collected at Fayetteville (94°W, 36°N), Arkansas.

Samples were collected on the roof

Table 1. Magnesium-28 in rain at Fayetteville (94°W, 36°N), Arkansas.

Date (1966)	Rain- fall (mm)	Sample size (liter)	Conc. of Mg ²⁸ (10 ⁻¹ atom/ ml)
16 July	31.5	685	1.7 ± 0.2
20 July	9.1	245	6.1 ± 0.7

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of the chemistry building of the University of Arkansas in five steel collectors, each having a surface area of 9×10^4 cm² and emptying into 30gallon polyethylene containers. Magnesium, strontium, barium, iron, lanthanum, lead, and bismuth carriers were added to the water sample. The magnesium fraction was isolated and exhaustively purified; the radiochemical procedure consisted of: (i) precipitation of ferric hydroxide with NaOH at pH 11, (ii) removal of Fe by precipitation of ferric hydroxide with NH₄Cl and NH_4OH at $pH \leq 7$, (iii) precipitation of the hydroxides of Mg, Sr, and Ba with NaOH, (iv) removal of Sr and Ba by the fuming nitric acid treatment, (v) precipitation of La with oxalic acid, (vi) precipitation of Pb and Bi as the sulfides, and (vii) ion-exchange separation of Mg from fission products such as zirconium or uranium with a column of anion-exchange resin AG 1 \times 8 (50 to 100 mesh, choride form). Magnesium was finally precipitated by the addition of NH_4Cl , $(NH_4)_2HPO_4$, and NH_4OH ; the precipitate was filtered, dried, weighed, and mounted, and betaradioactivity was determined. The chemical yield was 40 to 50 percent. Tracerlab omni-guard low-background counting system (background about 1 count/min) was used for the radioactivity measurements.

Experimental results are shown in Table 1 and Fig. 1. In one rain sample, a small tail (0.5 count/min) was observed after the decay of Mg28. Identification of the contaminant causing this tail was impossible, however, because of the extremely low activity.

Lack of data for the spallation reactions with targets of mass about 40 makes it impossible to calculate the production ratio of Mg28 and P32 from atmospheric argon. However, a crude estimate can be made for this ratio by using available experimental data for targets with somewhat larger masses.

Rudstam (5) gave the following empirical equation for the formation crosssection $\sigma(A_i, Z_i)$ of nuclides in a particular spallation group for the target of mass lying between 50 and 75:

$$\frac{\ln \sigma (A_i, Z_i)}{= P A_i - Q - R (Z_i - S A_i)^2} \quad (1)$$

where A_i and Z_i are mass number and atomic number of the nuclide produced, respectively, and P, Q, R, and S are constants.

The radionuclides such as Mg²⁸, P³², P³³, and so forth, are produced mainly from interactions of neutrons with ener-

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Fig. 1. Beta-radioactivity of the magnesium fraction isolated from rain. Slopes of the solid and dashed straight lines connecting the Mg^{28} activities in the 16 July and 20 July rains, respectively, correspond to a half-life of approximately 20 hours. This is in agreement with the 21.3-hour half-life of Mg²⁸.

gies of few hundred million electron volts with atmospheric argon, with some contribution from proton interactions. Values of P, Q, R, and S remain fairly constant with changes in neutron and proton energies; also, they are not greatly affected by the mass number and atomic number of the target nucleus.

According to Rudstam (see 5), average values of P, Q, R, and S in the bombardment of copper with 400 Mev neutrons and 60 and 340 Mev protons are: P, 0.521 ± 0.067 ; Q, 29.80 ± 3.77 ; R, 1.34 ± 0.24 ; and S, 0.469. If these values are introduced into Eq. 1, we have

$\sigma(Mg^{28})/\sigma(P^{32}) = 0.025 \pm 0.003$

The experimentally observed values of σ (Mg²⁸) $/\sigma$ (P³²) in the bombardment of copper by 2.2 Gev protons (6) and 5.7 Gev protons (7) are 0.06 and 0.08, respectively. This indicates that the value of production ratio calculated above is of the right order of magnitude.

According to Peters (1), an average rain contains 40 atoms of P³² per milliliter. Rao et al. (8) found the average concentration of P³² in rain to be about 50 atoms/ml at Fayetteville (94°W, 36°N), Arkansas. The following relation should hold between the concentrations of P³² and Mg²⁸:

$$C_{2s} = C_{32} \cdot \frac{\sigma(Mg^{2s})}{\sigma(P^{32})} \cdot \frac{\lambda_{32} + k_T}{\lambda_{2s} + k_T} \qquad (2)$$

where k_T is the reciprocal of the mean tropospheric residence time of the cosmic-ray-produced nuclides, and λ_{28} and λ_{32} are the decay constants of Mg²⁸

and P³². Unfortunately, the P³² contents in the rain samples used in this work are unknown, but if we assume that these samples contained about 50 atoms of P32 per milliliter, and use a value of $1/k_T = 30$ days for the tropospheric residence time, C₂₈ is calculated to be 0.12 atom of Mg²⁸ per milliliter. This value is in excellent agreement with the observed value of 0.17 atom of Mg²⁸ per milliliter for the 16 July 1966 rain. The Mg²⁸ concentration in the 20 July 1966 rain is higher than this by a factor of 3, but in view of the uncertainties involved in (i) the theoretical calculations, (ii) variations of the radionuclide concentrations from one rain to another, and also (iii) the tropospheric residence time, the above agreement has to be considered as excellent.

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