References and Notes

1. S. H. Kim, G. J. Quigley, F. L. Suddath, A. McPherson, D. Sneden, J. J. Kim, J. Weinzierl, A. Rich, *Science* **179**, 285 (1973).
2. The abbreviations used and notations are as follows: A, adenosine; T, thymidine; G, guanosine; C, cytidine; U, uridine; D, dihydrouridine; Ψ, pseudouridine; Y, a purine nucleoside; and m_1, methyl, and m_2, dimethyl (positions of these are noted by superscripts). Positions of the bases are indicated by the numbers placed after the name of the base.
3. A number of tRNA sequences are found in R. Holmquist, T. H. Jukes, S. Pangburn, *J. Mol. Biol.* **78**, 91 (1973).
4. F. L. Suddath *et al., Nature (Lond.)* **248**, 20 (1974).
5. S. H. Kim, G. Quigley, F. L. Suddath, A. McPherson, D. Sneden, J. J. Kim, J. Weinzierl, A. Rich, *J. Mol. Biol.* **75**, 421 (1973); S. H. Kim, G. J. Quigley, F. L. Suddath, A. McPherson, D. Sneden, J. J. Kim, J. Weinzierl, P. Blattmann, A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3746 (1972).
6. U. L. Rajbhandary and S. H. Chang, *J. Biol. Chem.* **243**, 598 (1968).
7. Chemical modifications are reviewed by: F. Cramer, *Prog. Nucleic Acid Res. Mol. Biol.* **11**, 391 (1971); S. E. Chang and D. Ish-Horowicz, *J. Mol. Biol.* **84**, 375 (1974).
8. S. H. Kim and A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **60**, 402 (1968).
9. M. Levitt, *Nature (Lond.)* **224**, 759 (1969).
10. M. Yaniv, A. Favre, B. G. Barrel, *ibid.* **223**, 1331 (1969).
11. I. Schwartz and J. Offengand, *Proc. Natl. Acad. Sci. U.S.A.*, in press.

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Hot Brines on Los Roques, Venezuela

Abstract. *Solar heating of dense natural brines to 47°C has been observed in a small lagoon in the Venezuelan Antilles and in an artificial brine reservoir in New York State. Calculations indicate that approximately 90 percent of the solar energy can be trapped in the brine by a combination of refraction and absorption. Brine ponds should be considered as solar energy collectors.*

Natural hot brines (as opposed to geothermal brines) have been found in a restricted shallow island lagoon in the Venezuelan Antilles. The dense brines result from the normal evaporation of seawater. They are covered by less dense, cooler natural seawater and freshwater.

The lagoon is located on Gran Roque or El Roque, the principal island in a group of islands known as Los Roques (see Fig. 1). This island group is located approximately 150 km north of Caracas, Venezuela. We visited the island in the early part of December 1973, about a week after the rainy season, when water levels were unusually high and the lagoons enlarged. Even the central part of the island, normally dry, was inundated with brackish water. The original purpose of our visit was to study the formation of lagoonal gypsum and of associated algal carbonates. However, we found that the bottom waters of the lagoon were uncomfortably hot (44° to 47°C) and were overlain by progressively cooler surface waters. Thus part of the study was directed toward this unusual thermal layering phenomenon.

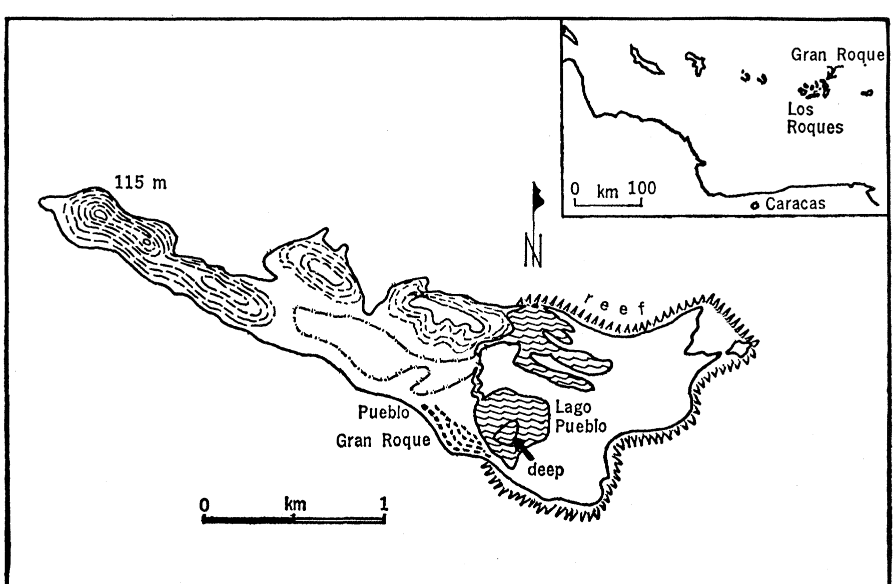

Fig. 1. Location map: outline of Gran Roque and of Lago Pueblo from aerial photographs.

The small village of Pueblo Gran Roque extends along the west coast of the island. The northern part of Gran Roque consists, in general, of a narrow ridge of metadiabase and metalamprophyre intruded by quartz diorite, granitic aplite, and pegmatite, which rises up to 115 m above sea level. The southeastern part of the island is flat and contains several connected small lagoons. The westernmost lagoon in the series, Lago Pueblo, contains the hot brines.

Lago Pueblo is roughly circular in outline and, on the average, less than 1 m deep. The unusual feature of the lagoon is a deep pool near its southwestern edge which was probed to a depth of 5.25 m. The water in the lagoon is derived to the largest extent from seawater inflow from the adjacent lagoons to the east but also in part from a freshwater stream draining the ridge to the northwest (*1*).

We measured the temperature of the water along a traverse from the eastern shore of the lagoon toward the deep by placing a thermometer at the desired level, allowing it to equilibrate, and then reading the temperature in place by means of a snorkel mask. Temperature readings at depths greater than 75 cm were difficult because of (i) the blurring of the water caused by thermal mixing, (ii) the extreme discomfort occasioned by the hot brine in the deeper parts, and (iii) the buoyancy effect of the dense brine. Samples of the water were taken at specific depths for the determination of densities and refractive indices.

The plot of temperature versus depth (Fig. 2) shows that the lagoon is divided into three distinct temperature regimes. The water from the surface to a depth of 27 cm was relatively cool, and the temperature increased gradually from 25° to 26.7°C. At 30 cm a thermocline was encountered in which the temperature gradient increases by 0.594°C per centimeter up to a maximum temperature of 43.3°C, encountered at depths over 53 cm. Both thermocline surfaces were visually observed at about 25 and 52 cm below the water surface and appeared as sharp, uneven planes in cross section.

At depths greater than 75 cm the temperature stabilized at 44.4°C.

The temperature measurements were made at around 11 a.m. Spot checking at 2 p.m. revealed that the temperature had risen to 47.2°C below the 53-cm depth; this was the new high temperature in all depths greater than 53 cm.

Temperature checks in other parts of the lake showed that the thermal layering was present over the entire lagoon. The deep part of the lagoon was dredged, and, after the metal dredging tool had been rapidly retrieved, it was found to be at the hot brine temperature. From this it would appear that the entire deep is filled with hot brine.

The density layering, determined in the laboratory by the pycnometer method, corresponded to the temperature layering and appeared to be about as abrupt (see Fig. 2). The density layering in the lagoon was seen in distinct layering of organic material suspended to a depth of about 20 to 23 cm. Below this level, the water was relatively clear, which may account for the clean bottom of purely chemical sediment (gypsum) in the shallow parts of the lagoon.

Some shallow water circulation was observed in the lagoon, probably caused by the dominant easterly winds. The circulation direction varied for the different density layers, but we did not study this feature in detail. The deep of the lagoon contained stagnant waters, as evidenced by the dredged-up black, sulfureted muds.

The presence of the hot brine in the lower water layers of the lagoon can be explained in terms of solar heating, which is due to a combination of refraction and absorption of the sun's energy. On the assumption that the average depth of the lagoon is 1 m, about 64 percent of the sun's energy would be absorbed if the water were perfectly clear (*2*). However, the brine layer was slightly murky, and presumably the absorption was greater. In addition, as the sun ray enters the lagoon, it becomes progressively refracted by the increasing density of the brine. Upon reaching the irregular, pale pink and white, highly reflective bottom, the light is scattered and reflected back in many directions and at many angles.

Figure 2 shows the refractive indices of water samples collected at various depths as determined with an Abbé refractometer. If these refractive indices

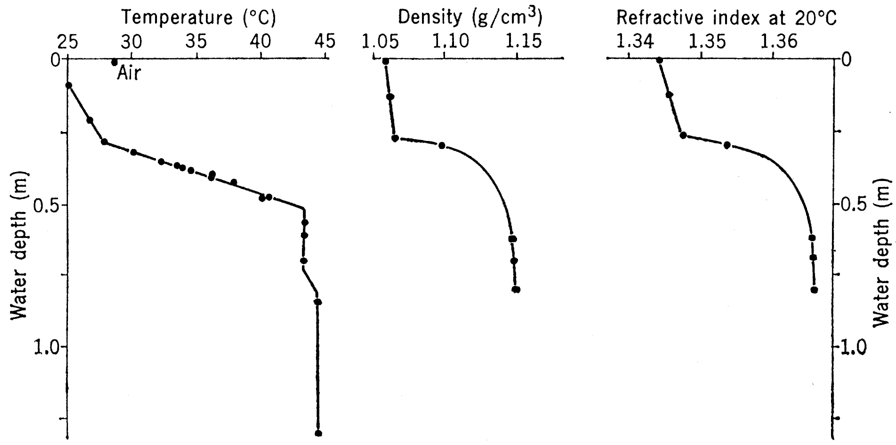

Fig. 2. Temperature, density, and refractive indices of brine samples collected in Lago Pueblo.

are introduced into Snell's formula, it can be calculated that all rays reflected from the irregular bottom at an angle of less than approximately 10° would remain trapped. This represents about 11 percent of the solar radiation received at the bottom, or about 4 percent of the solar energy entering the surface waters. In view of the length of travel of these low-angle rays, they would become totally absorbed by the brine. The remaining 32 percent of the solar energy ($100 - 64 - 4 = 32$ percent) is reflected from the bottom (assuming no bottom absorption) and travels upward through an average of 1 m of brine. About 64 percent of the above energy is also absorbed by the brine. Thus, it is estimated that, of the total solar energy entering the lagoon, almost 90 percent is absorbed. The total solar radiation received on a cloudless day at this latitude (*3*) is approximately 300 British thermal units per hour per square foot (7.024 kg cal m^{-2} $hour^{-1}$). Lago Pueblo thus can trap up to 79 kg cal m^{-2} day^{-1} in the summer rainy season and slightly less during the rains preceding the winter solstice, averaging about 15×10^6 kg cal day^{-1} for the whole lagoon.

The rainy season in December 1973 accompanied by severe storm tides had caused an unusually high water level in the lagoon. The surface layer consisted of seawater and freshwater of low enough salinity to allow normal sea life such as minnows to thrive. On the occasion of two other visits to Lago Pueblo in the past 2 years by Sonnenfeld, the water level was low and there was no fresh seawater covering the upper surface; halite was precipitating on the windward shores. On these two occasions the water temperature of all lagoon water was a constant 7° to 8°C above the ambient daily air temperature.

Such a thermocline was first reported in natural density-stratified lakes in Somaliland (*4*) and later in Washington State (*5*). Several lakes in Antarctica show the same phenomenon: At Lake Vanda relatively freshwater at a temperature of 0°C was found under 3.5 to 4 m of ice; at a depth of 66 m, the temperature was 25.6°C and the salinity was 1.1 g/cm^3 (*6*). At nearby Lake Bonney a temperature of only 8°C was recorded at a depth of 30 m (*7*).

In the fall of 1968, Hudec noted similar heating in an artificial brine reservoir in the southern part of New York State. The reservoir was used in conjunction with the storage of liquid propane in the salt beds. A scuba-diving geologist (*8*) reported the following temperature and density stratification for this reservoir: 15 to 20 cm of relatively fresh, cool surface water was separated by a sharp density change from progressively warmer brine occurring down to a second sharp density change at a depth of 140 to 170 cm. The dense brine temperature in the bottom 60 to 90 cm was estimated at more than 50°C, too hot for the human body to endure. The hot brine partially melted the bituminous lining of the reservoir floor.

Solar ponds in Israel are another example of artificial reservoirs: they are lined with a black bottom (for maximum absorption) and are about 1 m deep, the first 50 cm filled with brine and the top with freshwater. The energy trapped in the brine is converted to electricity (*9*). An example of a freshwater reservoir thermocline was reported in California (*10*).

In all of these cases, solar heating occurs only when the water is segregated into density layers of sufficient contrast. The density of the brine even when hot is greater than the density of cooler freshwater; thus the density profile remains constant. The freshwater layer provides insulation to the hot brine, conserving the heat.

The chemistry of the hot natural brines as distinct from geothermal brines is not as yet well understood. The unusually hot temperatures that are generated can stimulate dissolution, recrystallization, and reconstitution of some bottom sediments. Preliminary examination of the uppermost 1.5 m of bottom sediments cored in Lago Pueblo indicates that secondary changes have occurred in places.

The density-stratified brines in natural or artificial reservoirs should be reconsidered as solar energy collectors. Even in temperate and polar latitudes (namely, the examples in New York State and Lake Vanda, Antarctica), appreciable solar energy can be trapped. The chief advantages of such collectors are their relatively low cost of construction and maintenance and the high efficiency of energy absorption.

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References and Notes

1. P. Sonnenfeld, *Second Latin Am. Geol. Congr. Trans.* (Caracas, Venezuela, Nov. 1973), summary, p. 128.
2. A. Defant, *Physical Oceanography* (Pergamon, New York, 1961), vol. 1, table 19.
3. F. A. Brooks and W. Miller, in *Introduction to the Utilization of Solar Energy*, A. M. Zarem and D. D. Erway, Eds. (McGraw-Hill, New York, 1963).
4. E. Fulda, *Z. Deut. Geol. Ges.* **79** (monthly reports), 70 (1928).

G. C. Anderson, *Limnol. Oceanogr.* **3**, 259 (1958).

6. A. T. Wilson and H. W. Wellman, *Nature (Lond.)* **196**, 1171 (1962).
7. T. G. L. Shirtcliffe and R. F. Benseman, *J. Geophys. Res.* **69**, 3355 (1964); *ibid.*, p. 5257.
8. W. E. Cutcliffe, personal communication.
9. C. L. Strong, *Sci. Am.* **224** (No. 6), 124 (1971).
10. F. K. Browland and G. Koop, *Bull. Am. Phys. Soc.* **17**, 1108 (abstr.) (1972).
11. We thank D. Goddard of the Marine Geology Branch, Venezuelan Ministry of Mines and Hydrocarbons, Caracas, for courtesies extended. This research has been supported under a grant from the National Research Council of Canada.

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Lysosomal and Microsomal Glucuronidase: Genetic Variant Alters Electrophoretic Mobility of Both Hydrolases

Abstract. *An electrophoretic variant of β-glucuronidase is present in certain inbred mouse lines. The variant simultaneously affects the mobility of the lysosomal and microsomal forms of the enzyme. The difference is inherited as a single gene mapping at the position of the structural gene on chromosome 5. This confirms that β-glucuronidase at both intracellular sites is coded by the same structural gene.*

Acid hydrolases function in cellular catabolism, and deficient activity has been associated with lysosomal storage diseases in man (*1*). Several acid hydrolases, such as β-glucuronidase, β-D-*N*-acetylhexosaminidase, β-galactosidase, α-galactosidase, aryl sulfatase, and acid phosphatase, exist in multiple molecular forms (isozymes) when separated by gel electrophoresis (*2–4*). Biochemical, immunological, and genetic evidence indicate that a genetic and structural relationship may exist between the isozymes of any one acid hydrolase (*5–7*).

Investigation of the relationships of the multiple forms of an acid hydrolase would be facilitated by the existence of electrophoretic variants. Such variants would aid in determining whether the isozymes of a specific acid hydrolase are (i) coded by separate structural genes or (ii) share a common gene product coded by a single structural gene.

In the mouse, β-glucuronidase (E.C. 3.2.1.31) is assocated with the lysosomal and microsomal subcellular fractions (*8*), and both forms can be separated by gel electrophoresis (*9*). The structural gene locus for murine β-glucuronidase has been established on chromosome 5 with the use of a heat labile mutation of the enzyme (*10*). We report an electrophoretic variant of β-glucuronidase in inbred strains of mice which simultaneously alters the mobility of the lysosomal and microsomal glucuronidase activities, confirming earlier biochemical evidence indicating that a single structural gene codes for both forms of the enzyme (*8*). We believe that this is the first genetic electrophoretic variant that alters the mobility of an enzyme associated with two different subcellular structures. Previously, an electrophoretic variant of lysosomal β-glucuronidase was suggested in a wild mouse, but no mention was made of an altered mobility of the microsomal form of the enzyme (*11*).

Vertical starch-gel electrophoresis separates β-glucuronidase into two distinct zones of activity (Fig. 1). The more anodal zone (L) is associated with the lysosomes, while the slower migrating zone (M) is associated with the microsomes (*9, 12*). Although lysosomal β-glucuronidase was expressed in all tissues examined (liver, kidney, spleen, lung, and brain), the expression of microsomal β-glucuronidase varied from tissue to tissue. Liver homogenates were chosen for electrophoretic and genetic studies since liver best expressed the L and M forms of the enzyme. A survey of 28 inbred strains of mice revealed two glucuronidase phenotypes, one of which expressed faster migrating L and M enzymes than the other phenotype. The anodally migrating phenotype (Fig. 1, channels 3 and 7) was observed in six strains: A/J, A/St, BALB/cJ, LG/J, SEA/GnJ, and SM/J. This phenotype has been designated GUS-A. The slower migrating L and M types (Fig. 1, channels 1 and 5), designated GUS-B, were observed in 23 strains: AU/SsJ, BUB/BmJ, CBA/J, C57BL/6J, C57BL/10J, C57BR/cdJ, C57c/Ha, C57L/J, C58/J, C3H/HeJ, CE/J, DBA/2J, LP/J, MA/J, P/J, PL/J, RF/J, SJL/J, ST/bJ, SWR/J, WB/ReCz, and 129/J. Inbred mouse lines were obtained from Jackson Laboratory, Bar Harbor, Maine (designated J), and Roswell Park Memorial Institute.

The structural gene for β-glucuronidase, formerly designated *G*, is now designated *Gus* (*12*). The alleles for the fast and slow phenotypes, respectively, are designated Gus^{a} and Gus^{b}, and give rise to three possible phenotypes, GUS-A, GUS-B, and GUS-AB, in mating experiments. The respective genotypes are Gus^{a}/Gus^{a}, Gus^{b}/Gus^{b}, and Gus^{a}/Gus^{b}.

In a mating between SM/J mice (GUS-A, Fig. 1, channels 3 and 7) and SWR/J mice (GUS-B, Fig. 1, channels 1 and 5), all F_1 progeny expressed a heterozygous phenotype (GUS-AB) consisting of a broad band of activity intermediate between the two parental types in both the lysosomal and microsomal forms of the enzyme (Fig. 1, channels 2 and 6). Such a phenotype