mains with the segments. The pieces have been examined by Kerr and Fryxell (*8*), who agree with our conclusion that these pieces represent tetraploid *Gossypium*. Comparison of the boll segments with modern bolls of *Gossypium hirsutum* L. cv. 'Deltapine' show no significant differences.

The earliest previous evidence of the use of cotton was the fragment of fabric and string reported from the excavation at Mohenjo-Daro, Pakistan (*9*). The cotton was the Old World *G. arboreum* which Hutchinson equated with *bengalense* cotton (*3*). Dated at about 3000 B.C., the fragments indicate a well-established knowledge of weaving.

In the New World, the cotton reported by Bird (*10*) from Huaca Prieta is *Gossypium barbadense,* one of the tetraploid species. Again, the workmanship indicates that the manufacture of textiles from cotton fiber was not crude but had advanced to a relatively sophisticated art. Cotton was probably well-established as a crop plant by this time.

The Coxcatlán Cave cotton boll fragments, clearly dated at about 5800 B.C., establish the knowledge of a tetraploid species of *Gossypium* among people who were developing a method of cultivation suited to the special climatic conditions under which they lived. However, it is only in the next archeological level (Coxcatlán phase) at about 5000 B.C. that the remains of a number of species clearly indicate the use of agricultural practices.

The fundamental significance of the Coxcatlán cotton boll fragments is that *Gossypium hirsutum* was present in North America at an archeologically early period and that it was well differentiated from the other tetraploid American species of *Gossypium*. Evidence for the time interval required for the establishment of a tetraploid species subsequent to initial hybridization is incomplete. It is not impossible that this time interval could be on the order of tens of years. If it must be assumed that all of the tetraploid American *Gossypium* species have been the products of hybridization of the same two parents (as the genetic background for these species seems to indicate), the time interval needed to establish the differences recognizable in distinguishing the species must be much longer than the time span during which cotton has been known to man. The original hybridization (or hybridizations) occurred long before man became aware of the use of cotton fibers. Since the tetraploid *Gossypium* species are restricted to America and the Hawaiian Islands, the parental stocks must have grown near one another in America, but have since become lost along with countless other species. Much additional knowledge assembled from future archeological excavations will undoubtedly unravel the story of the use of cotton fibers, but the parental stocks contributing to the original hybridization may never be found.

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3 January 1964

Isotopic Molecules: Separation by Recycle Gas Chromatography

Abstract. *Gas chromatographic columns, greatly extended in length by the use of paired columns in a recycling apparatus, have been used to separate butane (n-C_4H_{10}) from deuterated butane (n-C_4D_{10}) and methane (CH_4) from deuterated methane (CD_4). The separation of monotritiated cyclobutane (C_4H_7T) from cyclobutane (C_4H_8) is nearly complete. This procedure is generally applicable to a wide variety of separations of isotopic molucules.*

The simple theory of gas chromatographic separation makes it quite apparent that increasing the column length under otherwise unaltered conditions results in better resolution of closely neighboring peaks (*1*). Indeed, A. J. P. Martin has often stated, "there is nothing excessive about a column a quarter of a mile long" (*2*). However, the technological problems of flow rates, overpressure, and so forth, have usually limited the lengths of packed columns to 15 or 30 m in most practical applications. Under these conditions, most isotopic molecules cannot be separated completely from one another, and isotopic separations by gas chromatography have been only infrequently reported (*3*). The only conspicuous exception has been the abundance of reports on the separation of H_2 and D_2, the *o*- and *p*- forms of H_2, and the various tritiated species (see *4*).

During the past 2 years, we have needed analytical techniques for separating a number of isotopic molecules, primarily hydrocarbons of low molecular weight such as CH_3T and CD_3T. We have worked out a recycling technique for extending the column length (*5*) almost indefinitely; this technique has proved applicable for separations of isotopic molecules, or for resolution of other molecular pairs that are difficult to separate on the usual columns.

The chromatographic system used in these experiments operates with two identical columns in series (C_1 and C_2 in Fig. 1) and a recorder in between (R_1). The columns are connected with four-way stopcocks (S_1 and S_2), for rapid reversal of the sequence of the columns in the series; and an additional recorder (R_2) is provided at the exit of the system. After injecting a sample containing a mixture of isotopic molecules, the isotopic mixture is allowed to proceed through C_1 and R_1 and into C_2. Almost all compounds with smaller retention volumes have by then passed

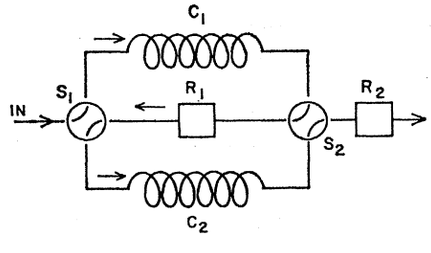

Fig. 1. Apparatus for recycle gas chromatography.

on through C_2 and R_2 and are no longer in the system. The stopcocks S_1 and S_2 are then both turned 90 deg in a counterclockwise direction. Any compounds that are near the end of C_1 will now be routed directly through R_2 and pass out of the system, while the mixture of isotopic molecules will pass through R_1 and back into C_1. The system is then cycled by alternating between the clockwise and counterclockwise configurations indefinitely until the isotopic molecules have distinctly different retention volumes, as shown by separated peaks in detector R_1, or until the peaks have broadened too much to be useful. Figure 2 shows the increased separation obtained with n-C_4H_{10} and n-C_4D_{10} by this recycling technique. The separation was quite good after one passage, but it continued to improve with further cycles; adequate peak shapes were maintained. Figure 2 shows the separation of CH_4 and CD_4, despite the almost complete failure of resolution in the first cycle (*6*).

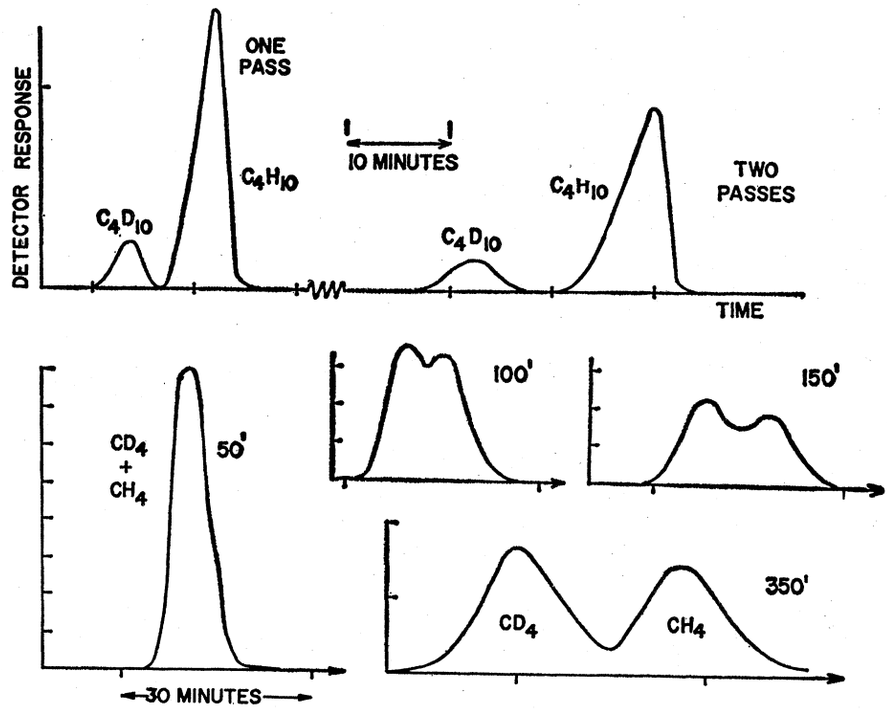

Fig. 2. Improved separation of isotopic hydrocarbons with recycling. (Top) n-C_4H_{10} and n-C_4D_{10}; (bottom) CH_4 and CD_4.

For the separation of CH_4 from CD_4, two 15-m columns consisting of copper tubing (inside diameter, 0.6 cm) were packed with 30 to 40 mesh 5A molecular sieve, and were operated at room temperature with an inlet pressure of 7.5 atm and an outlet pressure of 1 atm. The gas flow rate was 5 ml/sec, and the time required for the passage through each 15-m column was approximately 2 hours. The molecular sieve was activated in the column by helium flowing at the rate of 1 ml/sec for 24 hours at 250°C. For the separation of n-C_4H_{10} from n-C_4D_{10}, two 15-m columns (0.8-cm copper tubing) of safrole (30 percent by weight) on 30 to 40 mesh Chromsorb PA were operated at 0°C, and 2.7 atm excess inlet pressure. The gas flow rate was 4 ml/sec, and the separation required approximately 2 hours for each 15-m cycle.

We have recently observed that the monotritiated species of a variety of hydrocarbons emerges sooner (about 1 to 2 percent in retention volume) than the corresponding fully protonated species in packed-column experiments with various stationary phases (*7*). The separation of monotritiated cyclobutane (cyclo-C_4H_7T) from cyclobutane (cyclo-C_4H_8) obtained from such retention volume differences in the recycling apparatus is shown in Fig. 3. The two isotopic molecules are not completely resolved, but are sufficiently separated to permit substantial increases in the specific radioactivity (tritium atoms per unit weight of total cyclobutane) by selective isolation of the material initially emerging from the column. For example, isolation of all of the material to the point marked A on Fig. 3 will result in an increase in specific activity of 40-fold (40 percent C_4H_7T with only 1 percent of C_4H_8). The behavior in the columns of the isotopic cyclobutane molecules is not comparable (different symmetry and different half-widths) and this fact alone, observed with shorter columns, can serve as a diagnostic hint that separation may eventually be achieved through recycling.

The separation of cyclo-C_4H_7T and C_4H_8 was carried out with two 15-m columns (0.6-cm copper tubing) of safrole (30 percent by weight) on 30 to 40 mesh firebrick, operated at 0°C and 1.3 atm excess inlet pressure. The gas flow rate was 0.57 ml/sec, requiring approximately 6 hours for each 15-m cycle. The mole ratio of cyclo-C_4H_7T to cyclo-C_4H_8 in this experiment was approximately 10^{-9}, with the total mass of C_4H_7T about 10^{-11} g. The C_4H_7T was detected through measurement of its radioactivity in an internal proportional counter immediately after detection at R_2; propane was added to the flow stream after R_2 to make a suitable mixture for gas-proportional counting of radioactivity (*8*). The cyclo-C_4H_7T peak was not detected in R_1, and its exact location was unknown until recycling was stopped and the molecules were permitted to pass through R_2 and the proportional counter. The tritium was produced through the He^3 (n,p)T nuclear reaction and entered cyclobutane by hot-atom substitution; this method insures that multitritiated molecules are present in negligible quantities ($C_4H_6T_2/C_4H_7T$ is about 10^{-10}). All other molecules were present in cubic-

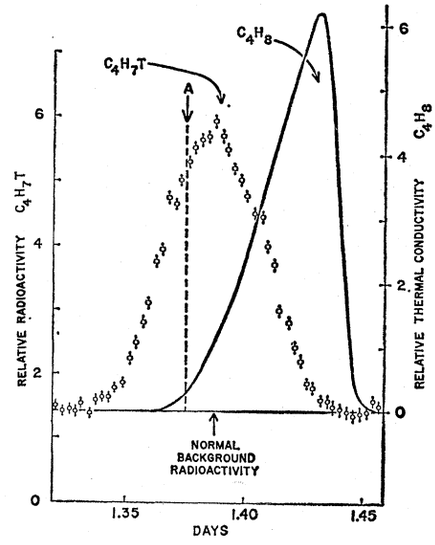

Fig. 3. Gas chromatographic separation of cyclo-C_4H_7T and C_4H_8.

centimeter (S.T.P.) quantities, and were measured with standard thermal conductivity equipment.

The most important general limitation on recycling indefinitely is reached when the material has spread and fills one entire section of the column. Thus, the separation of Fig. 3 could be continued until this point is reached; alternatively, the front of the peak to point *A* can be isolated, removed, and reinjected for a second passage. The separations reported here are typical, but optimum operating conditions for separation have not yet been systematically determined in any system. The technique has been used for 10- to 20-ml gas samples with the present equipment.

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Dolomitization: Observations on the Island of Bonaire, Netherlands Antilles

Abstract. *Evaporation of sea water and gypsum precipitation on south Bonaire produce dense brines having a high ratio of magnesium to calcium. Recent dolomite is a common replacement product in the associated carbonate sediments. A mass balance indicates that brine is being refluxed downward today, and the Pleistocene dolomite on north Bonaire could have been produced by similar reflux of brine.*

The problem of dolomitization of limestones [replacement of $CaCO_3$ by $CaMg(CO_3)_2$] has intrigued geologists for the last 100 years. Although many mechanisms have been proposed to explain dolomitization, no mechanism has yet been generally agreed upon. Observations which we recently made on the island of Bonaire in the Netherlands Antilles are consistent with the mechanism proposed to explain the dolomite found in the Permian Reef Complex (*1*).

In this proposed mechanism, the evaporation of sea water results in the precipitation of gypsum, which raises the ratio of magnesium to calcium of the water. The magnesium-rich water is capable of dolomitizing limestone and flows downward through the underlying sediments because it is more dense than fresh water or normal sea water. A similar mechanism has been proposed by Adams and Rhodes (*2*).

The south end of the island of Bonaire is a flat terrain close to sea level but isolated from the sea by a low coral rubble ridge. Exposed flats of soft, wet, Recent carbonate sediments cover about 34 m^2, and a number of hypersaline lakes are enclosed in the area by the coral rubble ridge. The sediments are composed of gypsum, aragonite, calcite, and dolomite. Most sediments from south Bonaire contain some dolomite, and some crusts are composed of as much as 95 percent of this mineral. Radiocarbon dating of two dolomite samples from these crusts gave ages of 1480 ± 140 and 2190 ± 150 years. The composition of the dolomite, determined by x-ray diffraction, ranges from $Mg_{46}Ca_{54}$ to $Mg_{44}Ca_{56}$. The ordering peaks 10·1, 10·5, and 20·1 of dolomite are visible on the x-ray powder pattern. The dolomite crystal size is about 2 microns, and replacement of shells demonstrates that at least some if not all of the dolomite was formed by replacing calcium carbonate.

Chemical analyses of the water in the hypersaline lakes and from pits in the Recent sediment indicate that the water has molar magnesium/calcium ratios which average about 30/1 compared with 5/1 in sea water. The results of a number of water analyses are plotted in Fig. 1. The logarithm of the chloride concentration is plotted against the logarithm of the concentration of the other ions. The data indicate that during evaporation of the original sea water, these waters have lost calcium carbonate and calcium sulfate, but there has been no detectable loss of magnesium. The water analyses are in general agreement with data of Usiglio (in Clarke, *3*) and confirm the gypsum solubility data of Posnjak (*4*) on gypsum.

Gypsum is a common mineral on the extensive flats of south Bonaire and is the predominant mineral in the sediments under the bottoms of the hypersaline lakes. On the other hand, the absence of halite and other late evaporite minerals in the sediments indicates

Table 1. Mass balance of Pekelmeer per unit area per year.

Source	g cm^{-2} $year^{-1}$					
	H_2O	Cl^-	$SO_4^=$	HCO_3^-	Mg^{++}	Ca^{++}
Sea water influx	85	1.6	0.23	0.012	.11	0.035
Rainfall	77					
Chemical precipitates	−0.015		−.07	−.005*		−.031
Evaporation	−150			−.005*		
Reflux	−12	−1.6	−.16	−.002	−.11	−.004

* $2HCO_3^- + Ca^{++} \rightarrow CaCO_3\downarrow + CO_2\uparrow + H_2O\uparrow$.