[Radiocarbon, Vol 25, No. 2, 1983, P 629-636]

# TIME HISTORY OF HUMAN GALLSTONES: APPLICATION OF THE POST-BOMB RADIOCARBON SIGNAL

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## ABSTRACT

Bomb-produced $^{14}C$ is a valuable tool for studying rates of short-term processes involving carbon cycling. This study shows that bomb $^{14}C$ is an excellent tracer of a biochemical process that takes place in the human body, namely the accretion of stones in the gallbladder. The methods developed for obtaining time histories of $^{14}C/^{12}C$ and $^{13}C/^{12}C$ in concentric layers from a large gallstone (30mm diameter) are reported. Formation times are assigned by matching the $^{14}C/^{12}C$ obtained from individual layers with those found for known-aged tree rings. Results show that the gallstone grew over a period of 10 years and seems to have lain dormant within the gallbladder for a period of 11 years. The average growth rate was 1.5mm/year.

## INTRODUCTION

The evolution of the pathogenesis of gallstones (GS) is divided into five stages (Small, 1974): metabolic, chemical, physical, growth, and symptomatic. In the first stage, the individual is predisposed to the formation of abnormal bile which then becomes supersaturated with cholesterol. Cholesterol crystals appear in the bile and a conglomeration of numerous crystals develops into a macroscopic stone. Finally, the stones give rise to symptoms or complications, such as jaundice or biliary colic, which cause the patient to seek medical attention. In order to interrupt or reverse the natural progression of cholelithiasis (gallstones), it is important to know the temporal relationship between the various stages.

The temporal history of GS was studied by Stenhouse (1979) and is studied here in a similar manner using the level of bomb-produced $^{14}C$ in individual concentric layers. Material that was accreted before the testing of thermonuclear bombs (pre-1955) contained ambient levels of $^{14}C$ ($\Delta^{14}C$ = -25 to 0°/oo). One of the three donors studied by Stenhouse (1979) had GS that were this old.

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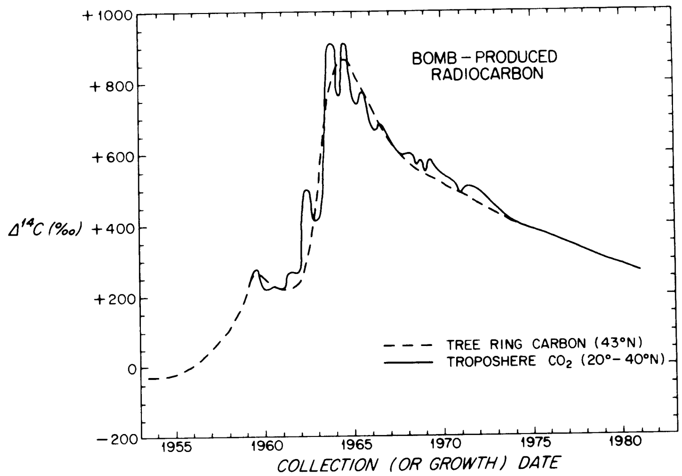


Fig 1. Post-bomb $\Delta^{14}C$ in tree rings (1950-1975)(Cain and Suess, 1976) and in atmospheric $CO_2$ (1960-1981)(Nydal, Löveseth, and Gulliksen, 1979; Levin, Munnich, and Weiss, 1980; Druffel, ms in preparation)

$^{14}C$ in tree rings as determined by Cain and Suess (1976) was used to assign dates to the growth layers. The curve in figure 1 represents $\Delta^{14}C$ values of annual rings obtained from a Bear Mountain oak from rural New York State (43°N, 74°W). The tree ring curve was preferred over measurements made directly on atmospheric $CO_2$, because of the large annual variations in atmospheric $^{14}C$ concentration observed between 1963 and 1973 due to the spring leak. It is of utmost importance, however, to address the offset observed between atmosphere/tree ring and contemporary human $^{14}C$ levels. Broecker, Schuler, and Olson (1959) found that it took 1 and 1.8 years before the $^{14}C$ concentration in blood and lung tissue, respectively, reached that in the atmosphere. Likewise, Nydal, Lövseth, and Syrstad (1971) examined human blood and hair samples over a long period (1963-1970) and concluded that bomb $^{14}C$ enters the body 1.4 years after production in the atmosphere. As cholesterol and bile pigments are the chief constituents in GS, the ages assigned to individual GS layers will be dependent upon the residence time for these chemicals in the body.

## APPROACH

The GS discussed below (No. XVIII) was obtained with the GS and common bile duct stones (CBS) from the Veterans Administration Hospital, San Diego, California (32°N). Only stones containing significant amounts of organic material (mostly cholesterol and bile pigments) were chosen. Stones were obtained only from patients who lived in the northern hemisphere and who never received radioisotopes.

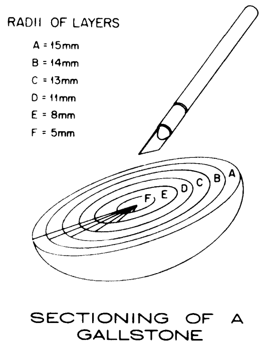


Fig 2. Schematic drawing showing the procedure for sectioning GS No. XVIII

The stone was washed in distilled water and dried at room temperature. It was cut in half using a jeweler's saw. Concentric layers were carefully sectioned with a scalpel, starting with the nucleus of the stone and working out toward the surface (fig 2). The diameter of the stone was $30.0 \pm 1$mm; the thickness of each of the 6 layers, was measured with an accuracy of $\pm$ 0.4mm.

At least 0.3g of material was needed per sample to generate a sufficient amount of $CO_2$ to analyze for $^{14}C$. Each sample was ground in a mortar and pestle in preparation for combustion. An aliquot of material was removed from some samples before combustion to determine per cent cholesterol (Fieser and Fieser, 1959). $^{14}C$ analyses were performed on extracted cholesterol from some samples other than the GS reported here. These measurements did not differ from those made on whole stones (Mok, Druffel, and Rampone, ms in preparation).

Combustion of each sample was performed by heating the material slowly in a flow of medical-grade oxygen ($80cm^3$/min, 8-10cm Hg). The combusted sample was passed through cupric oxide at 600°C to convert CO to $CO_2$, and bubbled through chromic acid to remove impurities. The sample was absorbed onto calcium oxide at 600°C, pumped free of contaminants at 400°C and then removed at 800°C. The purified $CO_2$ was passed through activated charcoal at 0°C to remove any residual contaminating gases.

All $CO_2$ samples were counted twice in a $100cm^3$ quartz and/or a $200cm^3$ copper counter at 900mm Hg and 25°C. Each sample was counted for a minimum of five days. All measurements were corrected for isotope fractionation to a

$\delta^{13}C$ of -25.0‰ relative to the PDB-1 standard. The standard used was 95% of the net count rate of NBS oxalic acid standard. All results are reported in terms of $\Delta^{14}C$, according to the Lamont normalization (Broecker and Olson, 1961).

Dates were assigned to layers by matching the $\Delta^{14}C$ value of each sample to the corresponding date using the $\Delta^{14}C$ curves for tree rings, human diet, and human blood (fig 3). It is possible to obtain two sets of dates for each stone, one set from each side of the bomb $^{14}C$ curve. We chose the set for which the oldest date corresponded to the nucleus and the most recent date corresponded to the outermost layers. Using the dates assigned to the nucleus and the outermost layer, we determined an average growth rate (both in d(radius)/dt and d(Volume)/dt) for the GS.

## RESULTS

Results for GS XVIII, obtained from an asymptomatic patient, are reviewed. Table 1 lists $^{14}C$ and stable isotopic measurements, as well as age assignments. All $\Delta^{14}C$ values found for this GS $<$+400‰. According to the composite $^{14}C$ curve in figure 3, this reflects growth before 1963 or after 1975. As the cholecystostomy (GS operation) was performed in March 1975, we concluded that growth took place during the earlier period. For comparison, the ages of the six GS layers are determined using each of the three $^{14}C$ curves shown in figure 3 (table 1).

The ages range from 1953.7-1962.0, 1955.6-1963.3 and 1955.6-1964.0 for the tree ring curve, dietary curve and blood curve, respectively. Despite an offset of 1-2 years among the three curves, the growth period of the GS determined using each of these three curves is the same, ca $8\pm1.5$ years. However, as these age assignments reflect the midpoint of each layer, assumptions must be made in order to calculate the growth period for the entire GS. First, a constant growth rate (d radius/d time) is postulated throughout the period of stone formation. In reality, the GS appears to have grown significantly faster during the formation of layers B and C. Second, we assume that the GS did not experience a hiatus at any point during its growth. Thus, assuming a constant growth rate of 1.5mm/yr, the growth period of the GS is determined (from tree ring curve) to have been from 1952.6 to 1962.3, representing a growth interval of ca 10 years. This estimate does not change significantly when either the diet or blood curves are used (fig 3).

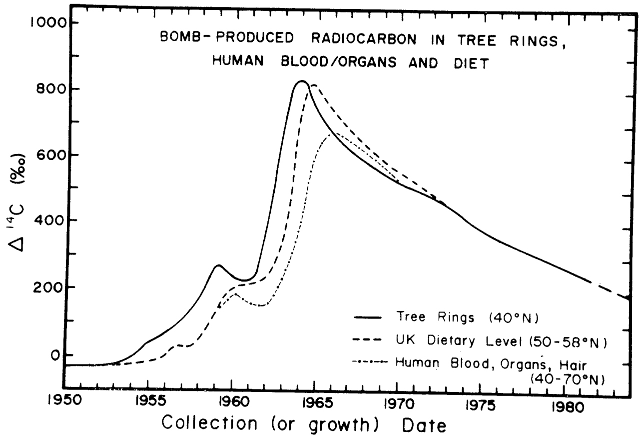


Fig 3. $\Delta^{14}C$ time histories in each of three carbon reservoirs: tree rings (Cain and Suess, 1976), computed dietary level for humans in the United Kingdom (Harkness and Walton, 1972; Stenhouse and Baxter, 1977) and composite human blood/organs (Broecker, Schuler, and Olson, 1959; Harkness and Walton, 1972; Nydal, Lövseth, and Syrstad, 1971).

These results indicate that the GS stopped growing from ca 1963 to the time of removal from the gallbladder (March 1975), a period of 11-12 years. This is an approximation of the period of no growth, as we cannot determine if the GS grew at a significantly reduced or accelerated rate during the formation of the outermost layer. Although we cannot totally dismiss the possibility of dissolution of other layers that may have formed subsequent to 1963, clinical evidence indicates that conditions favorable to dissolution of stones are not easily achieved in the gallbladder (Wolpers, 1968).

Figure 4 shows a positive correlation between $\delta^{13}C$ (table 1) and distance of the GS layers from the center. The values range from -19.1°/oo in the nucleus to -17.4°/oo in the outermost layer. This may indicate that bile pigment , which was enriched in the inner layers, has a significantly lighter $\delta^{13}C$ signature than cholesterol. Another possibility could be higher concentrations of calcium carbonate (Bills and Lewis, 1975), a mineral enriched in $^{13}C$ compared to both bile and cholesterol, in the outer layers.

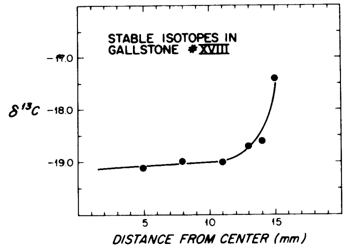


Fig 4. $\delta^{13}C$ results of GS layers as a function of distance from the center

TABLE 1. $\Delta^{14}C$, stable isotope measurements, and corresponding growth years of individual growth layers of GS XVIII

| Sample | Layer | Thickness of layer (mm) | $\delta^{13}C$ (°/oo) | $\Delta^{14}C$ (°/oo) | Growth years* Tree ring | Diet | Blood/organs |
|---|---|---|---|---|---|---|---|
| LJ-4178 | A (outermost) | 1 | -17.4 | +390±10 | 1962.0±.2 | 1963.1±.4 | 1964.0±.4 |
| -4179 | B | 1 | -18.6 | +128±25 | 1957.2±.3 | 1959.0±.4 | 1959.0±.4 |
| -4180 | C | 2 | -18.7 | +110±25 | 1956.8±.4 | 1958.8±.7 | 1958.8±.7 |
| -4181 | D | 3 | -19.0 | +100±25 | 1956.6±.4 | 1958.6±.7 | 1958.6±.7 |
| -4182 | E | 3 | -19.0 | -5±25 | 1953.7±1.2 | 1955.6±1.2 | 1955.6±1.2 |
| -4183 | F (nucleus) | 5 | -19.1 | +30±15 | 1954.7±.5 | 1957.0±1.5 | 1957.0±1.5 |

*From figure 3. Errors (in years) are determined from curves in figure 3 using ±1σ counting error reported with $\Delta^{14}C$ results in previous column.

## CONCLUSIONS

Chronology of cholelithiasis can now be determined using the level of bomb-produced $^{14}C$ in concentric layers of the stone. The growth period of the GS examined in this work spanned ca 10 years and seems to have lain dormant within the gallbladder for ca 10-12 years. The average growth rate of this stone was 1.5mm/year (or 1.6cm$^3$/yr). Further studies that include $^{14}C$ results for GS from symptomatic patients are needed for determining the progression of GS formation from the asymptomatic to the symptomatic stage.

ACKNOWLEDGMENTS

E M Druffel made isotopic measurements at the Mount Soledad Radiocarbon Laboratory, UCSD. Our thanks go to the patients who donated their gallstones for this study, including H E Suess. The technical skills of W Rampone, M Stenhouse, M Harvey, T W Linick, and G Calta are gratefully acknowledged. Our thanks are also given to an anonymous reviewer who contributed greatly to our interpretation. This work was supported by NSF Grant Nos. EAR 78-15183 and OCE 81-11954. This is Woods Hole Oceanographic Contribution No. 5185.

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