the twisting of the protofilaments: R. H. Crepeau *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1406 (1981); S. J. Edelstein, *J. Mol. Biol.* **150**, 557 (1981); E. A. Padlan and W. E. Love, *J. Biol. Chem.* **260**, 8280 (1985).

14. These models assume that the only distortion that occurs on binding of a protofilament to an aggregate occurs within the protofilament being bound. In fact, whenever a protofilament is stretched on binding to an aggregate, the aggregate will exhibit a slight compression and untwisting (see Fig. 1). These distortions are distributed among all of the protofilaments within the aggregate and are consequently small. In the analysis of the HbS aggregates in this report we have not attempted to take them into account. Nevertheless, in some cases they may be important. The observation of fibrous aggregates of limited radial extent indicates that the torsional rigidity of the fibers is sufficient to maintain twisting against the torque applied by the stretching of protofilaments on the surface of the aggregate.
15. The twisted crystals have a much larger *p/r* ratio than the other aggregates. Since there is no detailed information on the molecular packing in twisted crystals it is difficult to assess this observation. However, if one assumes that they are made up of double strands interacting side-to-side, there are three possible explanations for the observation: (i) the twisted crystals are not in equilibrium and are still growing radially; the balance of energy between binding energy and energy of stretching is different in the twisted crystals, so that (ii) there is less binding energy available for stretching the double strands; or (iii) the double strands with very long pitch are more rigid and require more energy to stretch. Observations (*4*) suggest that the twisted crystals are metastable intermediates of much larger aggregates and that (i) above is likely to be correct.
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Petroleum Associated with Polymetallic Sulfide in Sediment from Gorda Ridge

KEITH A. KVENVOLDEN, JOHN B. RAPP, FRANCES D. HOSTETTLER, JANET L. MORTON, J. DAVID KING, GEORGE E. CLAYPOOL

A sediment sample, impregnated with asphaltic petroleum and polymetallic sulfide, was dredged from the southern end of Gorda Ridge (the Escanaba Trough) off northern California, within the offshore Exclusive Economic Zone of the United States. The molecular distributions of hydrocarbons in this petroleum show that it was probably derived from terrestrial organic matter in turbidite sediment filling the Escanaba Trough. Hydrothermal activity at the Gorda Ridge spreading center provided the heat for petroleum formation and was the source of fluids for sulfide mineralization.

GORDA RIDGE IS AN ACTIVE OCEANic spreading center about 300 km long, bounded on the north and south, respectively, by the Blanco and Mendocino fracture zones (Fig. 1). The southern 90-km end of the ridge, referred to as the Escanaba Trough, is filled with as much as 500 m of Quaternary turbidite sediment (*1*). Within the trough, discrete volcanic centers have uplifted and in some places pierced the trough-filling turbidites (*2*). Siltstone slabs, basaltic glass, and massive polymetallic sulfide (*3*) have been dredged from the flank of one of these volcanic centers at latitude 40°45′N (Fig. 1). One of the samples is unusual in that it is a sediment impregnated with asphaltic petroleum and polymetallic sulfide. The association of petroleum and sulfide is of particular interest, because the sample was recovered within 200 nautical miles (370 km) of the coast of northern California and thus is within the Exclusive Economic Zone of the United States (*4*). The discovery is important with regard to offshore energy and mineral resources.

Evidence for hydrothermal activity at the Escanaba Trough comes from dredge samples obtained in 1983 that contained altered basaltic lava, manganese oxide crusts, nontronite, and sulfide (*5*). After a seismic-reflection survey of the Escanaba Trough in 1985, dredging at station 32 (Fig. 1) recovered 4.5 kg of massive polymetallic sulfide. The sulfide consists mainly of pyrrhotite with minor amounts of sphalerite, chalcopyrite, and galena (*3*); the unoxidized nature of the sulfide minerals indicates that the material was recovered from an actively forming hydrothermal deposit. Similar sulfide deposits have been found around active hot springs on the sediment-covered floor of Guaymas Basin in the Gulf of California (*6*).

Petroleum-like material was first noticed when the sulfide samples were being rinsed with acetone to remove water during the preparation of thin sections; one sample, weighing 185 g, turned the acetone dark brown. An analysis of this sample yielded an organic carbon content of 5.6% by weight, most of which was soluble in methylene chloride. Chromatography and mass spectrometry, both adapted from (*7*), were used to characterize the organic matter. The results of liquid-solid chromatography showed that the extractable organic material in this sample (55 mg/g) is an asphaltic petroleum composed of 2% aliphatic hydrocarbons, 44% aromatic hydrocarbons, and 54% nonhydrocarbons.

The aliphatic and aromatic hydrocarbons were fractionated and identified by gas-liquid chromatography and mass spectrometry (*7*). The following molecular markers were found: (i) a homologous series of *n*-alkanes (n-C_{14} to n-C_{40}) (Fig. 2A); (ii) isoprenoid hydrocarbons i-16, i-18, i-19 (pristane), and i-20 (phytane) (Fig. 2A); (iii) a series of 17α(H),21β(H) hopanes (C_{27} to C_{35} without C_{28}) including diastereomeric pairs of homohopanes (C_{31} to C_{35}) where *22R* dominates *22S* (average $22S/22R = 0.8$) (Fig. 2B); (iv) a series of 17β(H),21α(H) moretanes (C_{29} to C_{31}) (Fig. 2B); (v) steranes (C_{27} to C_{29}) with the 5α(H),14α(H),17α(H)-20*R* isomers more abundant than the 20*S* isomers and low amounts of the 5α(H),14β(H),17β(H)-20*R* and 20*S* diastereomers and rearranged steranes; (vi) a complex mixture of triaromatic steroids (C_{20} and C_{21}) and no monoaromatic steroids (*8*); and (vii) a mixture of polycyclic aromatic hydrocarbons (PAH) containing more nonalkylated than alkylated species (Fig. 2C). No sulfur-containing aromatic hydrocarbons were identified as major components of the aromatic fraction; organic sulfur is probably present, however, in the nonhydrocarbon fraction. All of the hydrocarbons identified are common in petroleum at various levels of maturity (*9*).

The original source of most of the hydrocarbons was probably the organic matter in the Pleistocene and younger turbidites of the Escanaba Trough. These terrigenous sediments, which have organic carbon contents ranging from 0.1 to 0.5% (*10*), derive mostly from the Klamath River and Columbia River drainage basins and were transported across the sea floor to the Escanaba Trough by turbidity currents (*11*). The *n*-alkanes larger than about n-C_{24} are characterized by the predominance of odd-carbon-number molecules with a carbon preference

K. A. Kvenvolden, J. B. Rapp, F. D. Hostettler, J. L. Morton, U.S. Geological Survey, Menlo Park, CA 94025.
J. D. King and G. E. Claypool, U.S. Geological Survey, Denver, CO 80225.

index (CPI$_{24-34}$) of 1.24 (*12*). This distribution is typical of an immature petroleum, and the dominance of odd-carbon-number molecules indicates contributions from terrigenous plant sources (*13*).

The distribution of hydrocarbons in the sample provides clues to their thermal history. For example, the small proportion of aliphatic hydrocarbons (2%) suggests that short-term heating was involved, because apparently the formation of these compounds requires significant time as well as elevated temperature (*13*). The pristane/phytane ratio of 1.7 is consistent with processes involving thermal alteration (*14*), as is the mixture of aromatic hydrocarbons (*15*). The isomerization of the 22*R* homohopanes and 20*R* steranes and the conversion of monoaromatic to triaromatic steroids yields information from which reaction temperatures can be estimated. The average ratio of 22*S*/22*R* homohopanes is 0.8, compared to an equilibrium ratio of about 1.2; only the 20*S*/20*R* ratio of the $\alpha\alpha\alpha C_{29}$ sterane could be measured with confidence, and this ratio is 0.3. The equilibrium ratio is about 1.0. Thus, the stereoisomers of hopanes and steranes in our sample have not reached equilibrium. In contrast, the monoaromatic steroids show complete conversion and cracking (only a complex mixture of C_{20} and C_{21} triaromatic steroids was found).

The kinetics of the isomerization and aromatization reactions have been evaluated (*16*); isomerization reactions are more time-dependent, whereas aromatization reactions are more temperature-dependent. The extent of these reactions observed in our sample is consistent with the formation of petroleum by intense heating of a short duration. These reactions probably have not been influenced by any mineral-matrix effects, because the large amount of organic material (5.6% organic carbon) tends to inhibit interactions between the organic compounds and the minerals. Using the methods of MacKenzie and McKenzie (*16*), we estimate that an effective time of heating and reaction of 10^4 years would require that the reactions occurred at 210°C, whereas an effective time of about 10^2 years would require temperatures of about 330°C. The petroleum must have formed rapidly when the terrestrial organic matter in the sediment was heated by the passage of hydrothermal fluids. The intimate association of polymetallic sulfide and petroleum suggests that hydrothermal fluids were responsible for the formation of the petroleum and its transportation to the sea floor. Quenching by or mixing with cold bottom water caused the sulfide and petroleum to precipitate.

The evidence suggests a recent origin for both the sulfide and the petroleum. For example, the sulfide minerals are unoxidized, indicating minimal exposure to seawater. The petroleum has not been biodegraded, as indicated by the dominant *n*-alkanes and the lack of a complex mixture of chromatographically unresolved aliphatic hydrocarbons (Fig. 2A), which are characteristic of biodegraded oils (*13*).

Support for our interpretations can be found in the well-documented studies of hydrothermal petroleum associated with sulfide in the Guaymas Basin, Gulf of California (*17*). Guaymas Basin is an active oceanic spreading center covered by a thick blanket of sediment. Our geochemical results on petroleum-bearing sulfide are comparable to those of Simoneit and Lonsdale (*17*), but there are some exceptions. For example, the *n*-alkanes in the Guaymas Basin sample range from *n*-C_{13} to *n*-C_{31} and have no significant odd-carbon-number dominance, whereas the *n*-alkanes *n*-C_{14} to *n*-C_{40} we observed have a marked odd-carbon-number dominance. This contrast may result from a difference in primary source material for the petroleum. The Guaymas Basin sediment contains mainly marine organic matter; in the Escanaba Trough the organic matter is predominantly terrestrial. The hydrocarbon mixtures from the Guaymas sample are much more complex, as demonstrated by an unresolved mix of compounds or "hump" on gas chromatograms, which was not observed in our sample. Other results are similar, however, including the asphaltic nature of both petroleum occurrences.

At present, the extent of petroleum-associated sulfide in sediment of the Escanaba Trough is unknown, because only one of the 25 specimens in the dredge contained petroleum. Thus the significance of our observation relative to any appraisal of possible economic resources is uncertain. Even if the occurrence of petroleum-associated sulfide

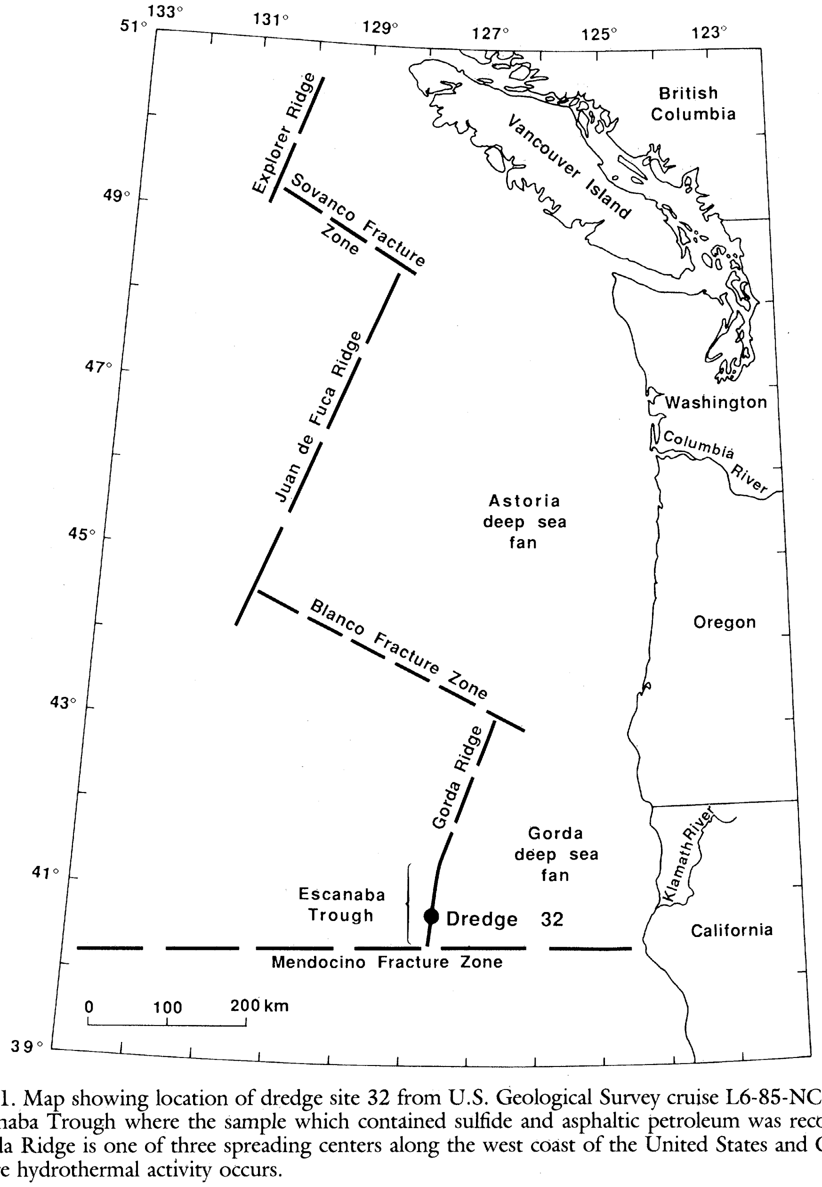

Fig. 1. Map showing location of dredge site 32 from U.S. Geological Survey cruise L6-85-NC to the Escanaba Trough where the sample which contained sulfide and asphaltic petroleum was recovered. Gorda Ridge is one of three spreading centers along the west coast of the United States and Canada where hydrothermal activity occurs.

at Escanaba Trough proves to be widespread, it is unlikely that the petroleum there will be a significant economic resource because (i) the dynamic geologic processes of an active spreading center do not permit formation of long-term traps for oil accumulation, (ii) the amount of organic matter within the trough sediment probably would not produce enough petroleum to be of economic interest, and (iii) the site is under about 3285 m of water, which is too deep for present petroleum production technology. Of much greater relevance than petroleum potential is the geochemical understanding of the details of processes that generate petroleum at oceanic spreading centers, and the recognition that active seepage of hydrothermally derived petroleum probably occurs in the submarine environment. That the petroleum-forming process, albeit by an unusual mechanism (hydrothermal activity), is taking place offshore within the economic jurisdiction of the United States suggests that further exploration is appropriate to extend knowledge of this phenomenon.

Note added in proof: During a U.S. Geological Survey cruise completed in August 1986, a second sample containing petroleum-like material was recovered near the same location (*3*).

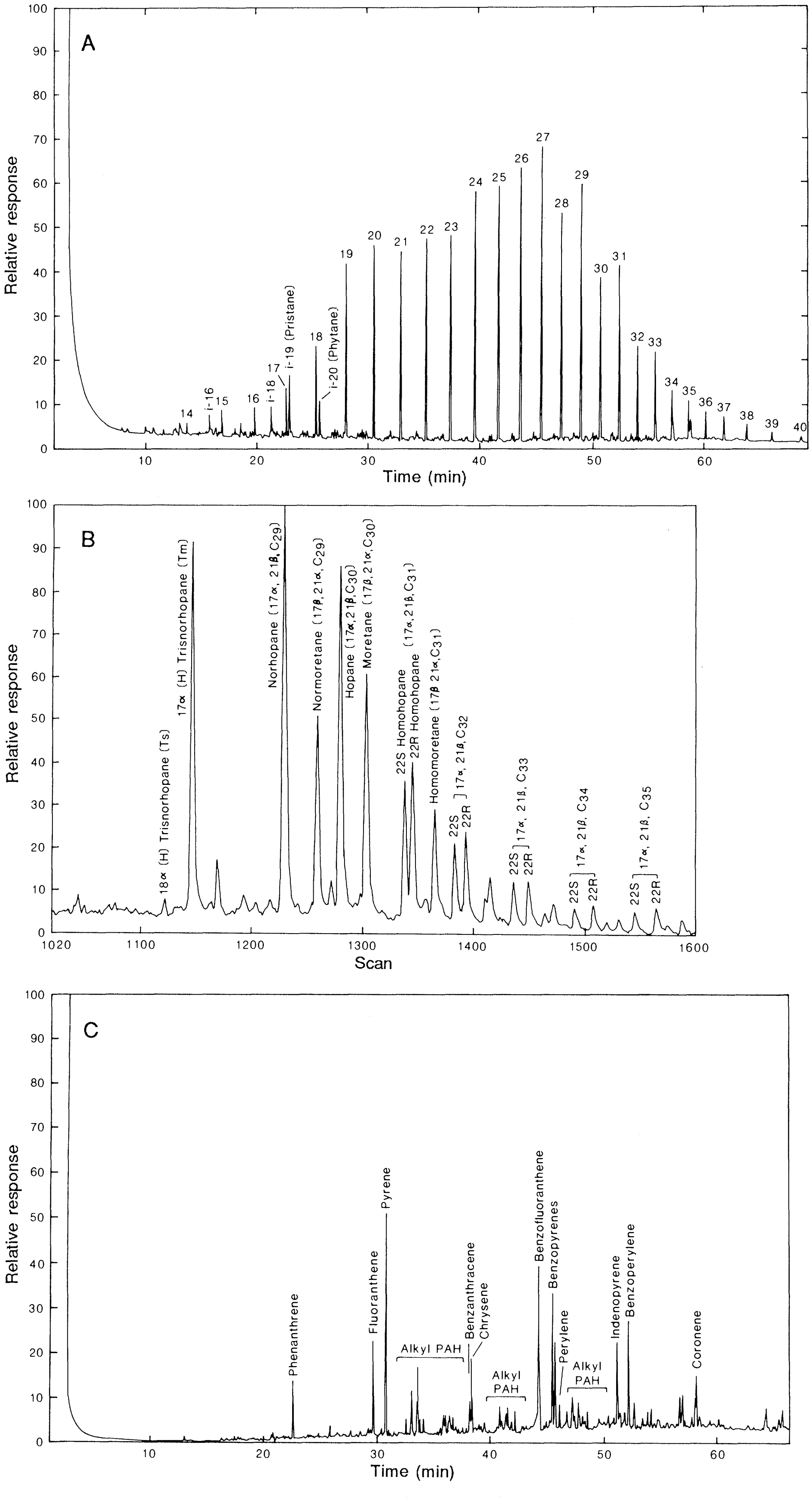

Fig. 2. Gas chromatographic and mass spectrometric evidence for hydrocarbons in asphaltic petroleum from the Escanaba Trough of the Gorda Ridge spreading center. (A) Gas chromatogram showing the distribution of aliphatic hydrocarbons, *n*-alkanes (identified by carbon number), and isoprenoids. (B) Mass fragmentogram (*m*/*z* = 191) showing the distribution of pentacyclic triterpanes. (C) Gas chromatogram of aromatic hydrocarbons.

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bonded phase, fused silica column). Single-ion monitoring was used to detect terpanes (mass-to-charge ratio m/z = 191) and steranes (m/z = 217). The use of trade names here and elsewhere in this report is for descriptive purposes only and does not constitute endorsement by the U.S. Geological Survey.
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Estrogen Memory Effect in Human Hepatocytes During Repeated Cell Division Without Hormone

S.-P. TAM, R. J. G. HACHÉ, R. G. DEELEY

Transient stimulation of target tissues by sex steroids can cause long-lasting changes that may facilitate or alter responses to subsequent hormonal treatment. How these altered characteristics are propagated during cell division in the absence of the stimulating hormone is unknown. The human hepatocarcinoma cell line HepG2 was used as a model to examine the effects of estrogen on the synthesis of serum apolipoproteins in vitro. Treatment with low concentrations of estrogen for 24 to 48 hours resulted in long-lasting alterations in the kinetics with which the cells responded to subsequent stimulation with estrogen. Manifestation of this memory effect was correlated quantitatively with the induction and propagation of a moderate-affinity, nuclear, estrogen-binding protein with the characteristics of a type II estrogen receptor. The data indicate that transient exposure of these cells to estrogen can induce changes in their response characteristics and composition of nuclear proteins that are inherited by daughter cells grown in the absence of hormone for more than ten generations.

MANY EFFECTS OF SEX STEROIDS on gene expression in their target tissues are dependent on the continued presence of hormone. However, in some cases, transient stimulation is sufficient to induce long-lasting alterations in the ability of the tissue to respond to subsequent hormonal treatment. Persistence of these altered response characteristics for several months, even in very young animals, has raised the possibility that this so-called priming or memory effect may not simply be long lasting, but might be propagated during growth of the tissue in the absence of the stimulating hormone. How this is accomplished is unknown. A particularly well-characterized example of priming has been provided by studies of vitellogenesis in avian and amphibian liver. Short-term primary stimulation of adult male or immature animals with estrogen results in transient activation of genes specifying major yolk proteins, such as vitellogenin, together with long-lasting alterations in the kinetics with which they respond to secondary stimulation (*1–4*). To date, this long-term effect of estrogen on hepatic gene expression has been shown to occur only in egg-laying vertebrates. We now report that (i) an estrogen-memory effect can be reproduced entirely in vitro in an established line of human hepatoma cells and (ii) manifestation of this effect after repeated cell divisions in the absence of hormone correlates quantitatively with the induction and propagation of a moderate-affinity, nuclear, estrogen-binding protein with characteristics of the type II estrogen receptor.

The human hepatoma line HepG2, which was established in culture by Knowles *et al.* (*5*), has been shown to retain many of the differentiated characteristics of normal hepatocytes, including the ability to synthesize the major serum apolipoproteins of hepatic origin. Because of the importance of these proteins in determining atherosclerotic risk, we examined the possibility of using these cells as an in vitro model to study hormonal regulation of their synthesis. Our studies revealed that estrogen has two quite distinct dose-dependent effects on the synthesis of these proteins (*6*). At concentrations in the physiological range, it induces increases in the levels of messenger RNA's (mRNA's) specifying apolipoproteins apoAI and apoCII (*7, 8*), both of which are components of nascent high-density lipoprotein particles. At 25- to 50-fold higher concentrations, the hormone increases synthesis of two additional apolipoproteins, apoE and apoB, which are major components of nascent very low density lipoproteins (VLDL's), by a mechanism that also involves alterations in apolipoprotein mRNA levels (*6*).

In addition to requiring different concentrations of hormone, induction of these two pairs of apolipoproteins was found to occur with different kinetics. Induction of apoB and apoE, unlike that of apoAI and apoCII, was characterized by a lag of 6 to 8 hours (*6*). However, if the cells were preincubated for 24 to 48 hours in estrogen at concentrations well below the threshold required to induce apoB and apoE, the lag in induction of these two proteins on exposure to high concentrations of the hormone could be eliminated. This difference between the response characteristics of control and pretreated cultures is reminiscent of differences between the primary and secondary induction kinetics of avian and amphibian vitellogenin genes. The observation prompted us to examine whether these human cells also display a memory effect similar to that observed in the liver of egg-laying animals. Since established lines of amphibian and avian hepatocytes do not exist, this also provided for the first time the opportunity to examine the propagation of such an effect in a homogeneous population of dividing cells.

Newly confluent cells were maintained for 48 hours in medium containing 17β-estradiol at an initial concentration of 20 nM. This treatment maximally induces apoAI and apoCII but does not alter the production of

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Department of Biochemistry, Queen's University, Kingston, Ontario, Canada K7L 3N6.