SAC-antibody-antigen complex, to sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. The standard ^{125}I-labeled α subunit was completely precipitated by the antiserum and could be recovered when the complex was disrupted (Fig. 2). Disruption of the complex yielded a single labeled protein that comigrated with highly purified α subunit. Furthermore, no newly synthesized protein was detected by the antiserum when excess unlabeled α subunit was included in the precipitation reaction.

Since we had found previously that fetal kidney, and to a lesser extent, liver, can synthesize and secrete the β subunit of hCG (*7*) and that both subunits occur in similar cell populations of the placenta, kidney, and liver (*8*), we examined the bioactivity of the hCG of fetal origin. We used a modification of the Van Damme mouse Leydig cell assay (*9*), in which human luteinizing hormone (hLH) and hCG stimulate testosterone production by dispersed cells. Incubation media and tissue homogenates were obtained as described, except that detergent was deleted; portions of each sample were added to the cultured cells and, after incubation, the culture medium was assayed for testosterone. Finally, the Leydig cells were washed, solubilized, and counted to determine the extent of binding of newly synthesized hormone.

Chorionic gonadotropin synthesized by placenta and kidney was found to bind to Leydig cells (Fig. 3). This binding of the labeled hormone was completely abolished by the addition of excess, unlabeled hCG, suggesting specific binding of the newly synthesized hormone to the cell surface. Both placental and fetal hCG was bioactive, as evidenced by Leydig cell testosterone production. It is of interest that the hCG of fetal kidney origin was more bioactive than the placenta-derived hormone. We reported previously that chorionic gonadotropin from the fetal kidney is significantly more bioactive than the hormone extracted from the placenta or from other fetal tissues (*6*). The possibility that hCG synthesized by the fetal kidney is conformationally distinct from other forms awaits further investigation.

Although the physiologic role of hCG past the first trimester remains obscure, the finding that the fetus synthesizes bioactive hormone during the second trimester suggests a possible regulatory function. Chorionic gonadotropin stimulates dehydroepiandrosterone sulfate production by the fetal zone of the fetal adrenal cortex (*10, 11*), and this zone is histologically normal into the second trimester in fetuses lacking ACTH (*12*). Similarly, anencephalic fetuses have histologically normal thyroid glands despite the lack of thyroid-stimulating hormone (*13*); fetal hCG appears to bind to microsomes prepared from the fetal thyroid (*14*) and may exert a trophic effect. In addition, it is well established that hCG can bind to the human fetal testis resulting in testosterone biosynthesis (*15*).

Taken together, these observations suggest that hCG may partially regulate fetal steroidogenesis and possibly influence the development or maintenance of tissue differentiation. The autoregulatory role of this hormone during human fetal development awaits further investigation.

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Mobilization of Cellular Calcium-45 and Lead-210: Effect of Physiological Stimuli

Abstract. *Isolated rat hepatocytes in primary culture were used as a model system to evaluate the effects of selected hormones and culture conditions on the efflux of calcium-45 and lead-210 from cells labeled with these isotopes. Alpha-adrenergic stimuli, angiotensin, vasopressin, dibutyryl adenosine 3′,5′-monophosphate, and reduced phosphate concentrations in the medium increased the efflux of calcium-45 and lead-210. Glucagon and insulin had no effect, but increased phosphate concentrations decreased the efflux of both isotopes. Experiments with hepatocytes cultured in a medium free of calcium and lead demonstrated that the increased efflux of calcium-45 and lead-210 induced by hormones was the result of mobilization of the ions from intracellular stores. The data indicate that the physiological stimuli that mobilized calcium ions also mobilized lead ions, and that the mobilized lead would be available to interact with calcium-mediated cell functions.*

Experimental and clinical studies show that the physiological regulators of calcium metabolism regulate systemic lead metabolism in a similar manner (*1*). However, the effects of the physiological regulators of cellular calcium metabolism on cellular lead metabolism have not been studied systematically. For the most part, lead and calcium are associated with mitochondria, and their subcellular distribution is similar in isolated cells (*2*) and synaptosomes (*3*). That the cellular metabolism of lead is similar to that of calcium is further supported by the observation that the energy-dependent uptake of Pb^{2+} by mitochondria shares many of the characteristics of the Ca^{2+} accumulation reaction (*4*).

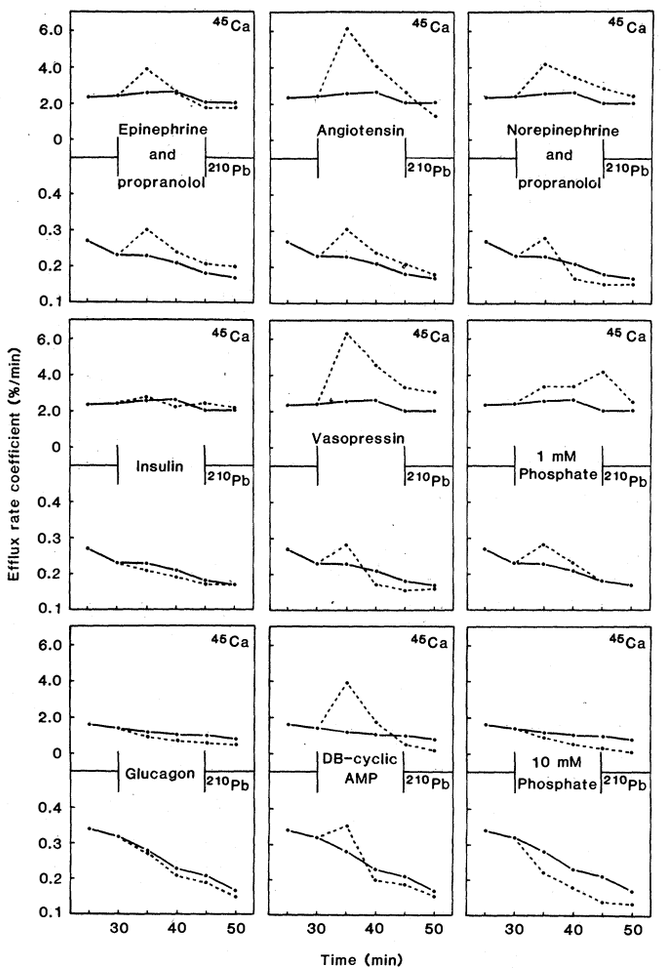
In our laboratory, rat hepatocytes in primary culture have been used as a model system to study cellular interactions of lead and calcium (*5*). The effect of hormones on cellular calcium metabolism may be studied by measuring changes in the rate of ^{45}Ca loss from cells labeled with this isotope (*6*). We conducted the experiments described herein in order to compare the mobilization of ^{210}Pb and ^{45}Ca from hepatocytes by physiological stimuli.

The effects of selected hormones and culture conditions known to affect the efflux of ^{45}Ca from labeled hepatocytes (*6*), or suspected of such activity, are presented in Fig. 1. α-Adrenergic agonists (epinephrine with propranolol and norepinephrine with propranolol), angiotensin, vasopressin, dibutyryl adenosine

3′,5′-monophosphate (dibutyryl cyclic AMP), and reduced phosphate concentrations increased the rate of efflux of ^{45}Ca and ^{210}Pb. Insulin and glucagon had no effect, but increased phosphate concentrations decreased the mobilization of lead and calcium. These data indicate that the cellular metabolism of lead and calcium is regulated by similar, but not necessarily identical, mechanisms. The increases in the rates of efflux of the isotopes may be due to (i) an increased rate of exchange across the cell membrane with no change in the cellular concentration, (ii) an increased rate of transport out of the cell accompanied by a decrease in the cellular concentration, (iii) an increased release of the isotope from intracellular stores, or (iv) some combination of the above.

To determine if the increase in ^{45}Ca and ^{210}Pb efflux was the result of an increased rate of ion exchange across the plasma membrane or the result of mobilization of the isotopes from internal cellular stores, we conducted experiments with hepatocytes cultured in calcium- and lead-free media (Fig. 2). Epinephrine with propranolol and vasopressin increased the efflux rate coefficient of both ^{210}Pb and ^{45}Ca, demonstrating an effect of intracellular events, not an increase in the rate of exchange across the plasma membrane. These experiments do not indicate whether the ^{45}Ca and ^{210}Pb were mobilized from the same intracellular site, nor whether an increased ^{210}Pb efflux rate coefficient is accompanied by an increase in cytosolic levels of Pb^{2+} as has been demonstrated for Ca^{2+} (*7*). Moreover, since the specific activity of the isotopes inside the cells is changing, and because we do not know the specific activity of the kinetic pools responsive to the physiological stimuli, no estimation of the mass of lead or calcium mobilized can be made.

The temporal and spatial distribution of cellular calcium concentrations as a coupling factor in calcium-mediated cell functions and the diversity of these functions have received considerable attention in recent years. The transient increase in the cytoplasmic concentration of Ca^{2+} which precedes the hormonal or electrical activation of calcium-mediated cell function is a result of the mobilization of Ca^{2+} from intracellular stores, for example, mitochondria, or a change in the permeability of the plasma membrane to Ca^{2+} (*7*). The transient increase in cytosolic concentrations of free Ca^{2+} following these stimuli permit the involvement of this ion in many biochemical and physiological phenomena. These phenomena—which include the regulation of cyclic nucleotide levels, cellular calcium metabolism, microfilaments and microtubules, carbohydrate metabolism, amino acid transport, neurotransmitter release, transduction of visual stimuli, cell proliferation, and cell death—may be vulnerable to lead intoxication (*8*). Our experiments emphasize the similarity between lead and calcium metabolism



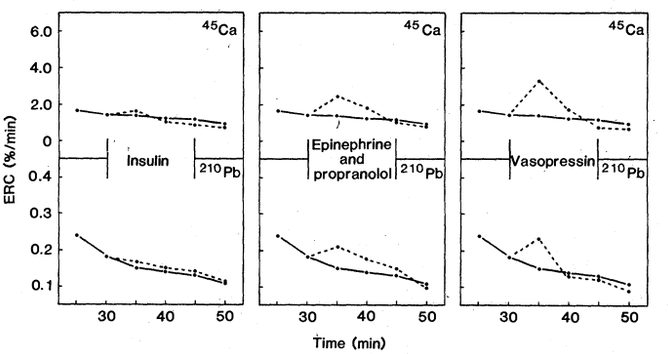


Fig. 1 (left). Effect of selected physiological stimuli on the efflux of ^{45}Ca and ^{210}Pb from isolated rat hepatocytes in primary culture. Isolated hepatocytes were prepared and cultured as described (*9*, *2*). The cells were labeled in complete Williams' medium E (Gibco) with 25 μCi of ^{45}Ca per milliliter (1.6 m*M* calcium) or 1 μCi of ^{210}Pb per milliliter (3 μ*M* lead; New England Nuclear) as described (*2*). After 24 hours of labeling, each culture was washed six times (0.5 minute each) with 0.25*M* sucrose in 10 m*M* Hepes buffer at *p*H 7.0 to remove the labeling medium (*2*, *5*). One milliliter of medium without radioisotope was added to each well at washout time, $t = 0$. At 5-minute intervals, 0.5-ml portions of medium were removed for scintillation counting and the remaining medium was aspirated and replaced with 1.0 ml of fresh medium. Between minutes 30 and 45 of desaturation, the steady-state efflux was perturbed by the inclusion of 10 μ*M* epinephrine plus 10 μ*M* propranolol, 10 μ*M* angiotensin, 10 μ*M* norepinephrine plus 10 μ*M* propranolol, 2 mU of insulin per milliliter, 10 μ*M* vasopressin, 1 m*M* phosphate, 2 μ*M* glucagon, 100 μ*M* dibutyryl cyclic AMP, or 10 m*M* phosphate in the washout medium. The efflux rate coefficient (*ERC*) was calculated as $ERC = DPM_w/[(DPM_c)_{mean}\ \Delta t] \times 100$, where DPM_w is the radioactivity lost from the cells to the washout medium during the interval Δt and $(DPM_c)_{mean}$ is the mean radioactivity left in the cells between time t and $t + \Delta t$ (*10*). The data represent the mean of triplicate cultures from a representative experiment. Solid lines, untreated control cells; broken lines, treated cells. Fig. 2 (above). Effect of calcium- and lead-free medium on the hormonal stimulation of ^{45}Ca and ^{210}Pb efflux from isolated rat hepatocytes in primary culture. Hepatocytes were prepared and labeled and the ERC calculated as described in Fig. 1, except that the desaturation of ^{45}Ca and ^{210}Pb was conducted in Ca^{2+}- and Pb^{2+}-free medium [minimum essential medium (MEM) amino acids, MEM nonessential amino acids, and MEM vitamins (Gibco), 10 m*M* Hepes buffer, 10 percent dialyzed fetal bovine serum, 2 mU of insulin per milliliter, 2 m*M* L-glutamine, 20 μg of gentamicin sulfate per milliliter, 120 m*M* NaCl, 5.4 m*M* KCl, 2 m*M* NaH_2PO_4, 1.7 m*M* $MgSO_4$, 0.3 μ*M* $Fe(NO_3)_3$, 0.6 μ*M* MnCl, and 25 m*M* $NaHCO_3$]. The data represent the mean of triplicate cultures from a representative experiment. Solid lines, untreated control cells; broken lines, treated cells.

at the cellular level, as well as the dynamic characteristic of intracellular lead in an isolated, intact cell system. The data indicate that lead may be mobilized by cellular stimuli that mobilize calcium, and we postulate that the mobilized lead may be available to interfere with, or participate in, the calcium-mediated cell functions elicited by these stimuli.

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Enchenopa binotata Complex: Sympatric Speciation?

Abstract. Enchenopa binotata *is a complex of six treehopper species that have diverged along host plant lines. When females were forced to oviposit on "adopted" host plants, few eggs were deposited. Fewer eggs hatched on "adopted" hosts and those that did hatch did so in response to the phenology of the "adopted" host. Mortality of nymphs on "adopted" hosts was substantially higher than on native hosts. These and other data support a sympatric model of speciation through shifts in host plants.*

Allopatric speciation was once considered the primary means by which populations differentiated into reproductively isolated species. This view has been challenged (*1*), and nonallopatric mechanisms have become more generally accepted. Bush (*2*) suggests that shifts in host plants promoted sympatric divergence of some phytophagous insects such as *Rhagoletis*. Futuyma and Mayer (*3*), after reviewing the evidence on *Rhagoletis*, accept the possibility of such a mechanism but conclude that there is little empirical evidence to support it. At issue is whether a host plant by itself can act as an effective reproductive barrier to limit gene flow after a host shift has occurred. We have recently presented data (*4–6*) which support a sympatric model of speciation by shifts in host plants.

Enchenopa binotata (Say) is a phytophagous insect that occurs from Panama throughout eastern North America. In North America it has a single generation per year and is found on six species of host plant. These hosts (*Ptelea trifoliata*, *Cercis canadensis*, *Juglans nigra*, *Viburnum prunifolium*, *Celastrus scandens*, and *Robinia pseudoacacia*) are evolutionarily diverse (*7*) and sympatric throughout the eastern United States. *Enchenopa binotata* has been considered a single polyphagous species. However, *Enchenopa* on each host differs in the coloration of nymphs, oviposition sites, nymphal feeding sites, seasonal and diurnal patterns of oviposition, and the number of eggs per egg mass. When females are given a choice of host plants on which to oviposit they select the host on which they were raised. When males and females from all hosts are confined to a single cage, there are few matings by insects of mixed host origin, and the length of mixed matings are considerably shorter. Even under conditions imposed by a cage, mating tends to occur on the host on which females were raised (*4–6*). Therefore, although we believe sympatric speciation via host plant shift does not require that females show a host preference, members of the *Enchenopa binotata* complex do demonstrate such a behavioral choice.

Allochronic life histories are important in maintaining reproductive isolation among members of this complex. Eggs hatch on each host (with the exception of *Cercis canadensis*) about the time the host is in flower. Allochronic egg hatch combined with differences in maturation produces temporal differences in mating; differences in the time of day that mating occurs further reduce the possibility of hybridization. Allochronic and diurnal differences in mating reproductively isolate adults from four of the six hosts. Members of the last pair are effectively isolated from each other by allochronic flight activity, which occurs about a week apart. On one host, almost all flight activity occurs before mating begins on that host; hence, there is very little flight by either sex once mating begins and virtually none after oviposition starts. Ovipositional attractants in egg froth tend to keep females on their hosts (*4–6*). In fact, movement by males and females throughout the summer, even among nearby conspecific hosts, is almost nonexistent (*8*).

Females only mate once; this should promote competition among males as the number of virgins decreases and increase male dispersal to new hosts. We have found that male flight activity does not increase as the number of virgins decreases. The lack of male dispersal stems from the inability of males to recognize mated females, high male mortality, and short male longevity. When mating is completed on a given host there are few or no males surviving (*4–6*).

Electrophoretically, *Enchenopa* from each host differ in the frequency and fixation of electromorphs even when collected from two adjacent tree species. There were even electrophoretic differences in the *Enchenopa* among individual conspecific trees located very close to each other. Genetic distances calculated from electrophoretic data indicate that *Enchenopa* on *J. nigra* diverged first; then divergences on *P. trifoliata*, *R. pseudoacacia*, *C. canadensis*, *V. prunifolium*, and *C. scandens* followed in the order given. Estimates of the time of divergence by means of the molecular time clock (*6*) suggest that speciation has been recent—that is, within the last 250,000 years. (In this estimate, selection is assumed to be absent.)