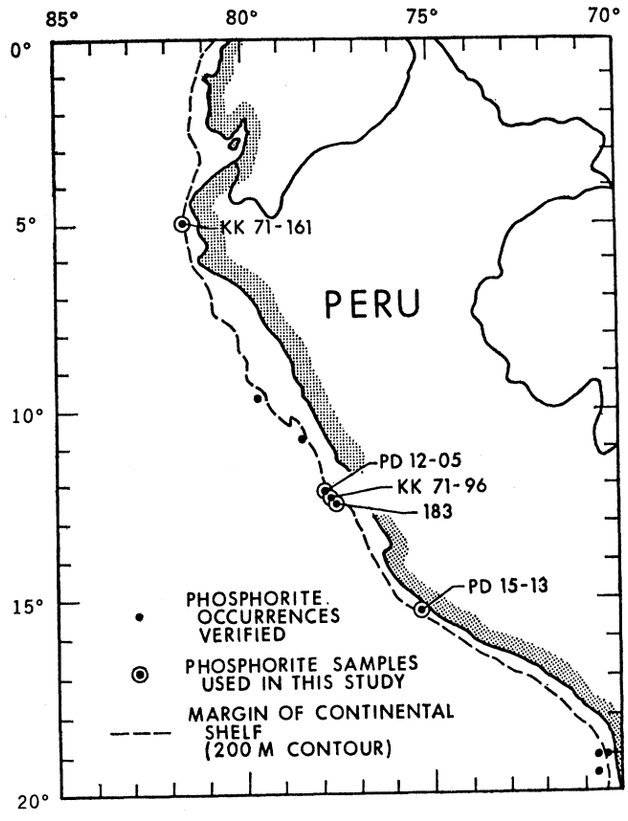

Fig. 1. Map showing phosphorite occurrences and sample locations on the continental margin of Peru.

300,000 years to Recent. This indicates that the phosphorite nodules off Peru are, at least in part, forming currently, in agreement with the results of Baturin *et al.* (*8*) for another location.

No absolute ages can be calculated from the $^{230}Th/^{234}U$ ratios because the ^{230}Th content in the phosphorites at the time of formation is not known. An upper limit can be placed on their ages, however, by assuming that all of the ^{230}Th measured is a decay product of the parent uranium in the nodules. These upper age limits range from 125,000 to 2000 years ago, which is further evidence for a late Pleistocene to Recent age for the phosphorites off Peru. The presence of measurable amounts of thorium in our samples (Table 1) suggests that some of the ^{230}Th found in the phosphorites could have an external origin, and hence the true ^{230}Th ages may be substantially lower than the upper limits.

It could be argued that the assumption of a closed system is invalid, and that the ages reported here are not real but apparent, due to secondary addition of uranium to the phosphorites. Secondary additions of uranium from seawater to pre-Pleistocene phosphorites with low initial uranium contents could result in $^{234}U/^{238}U$ ratios between 1.00 and 1.15 and $^{230}Th/^{234}Th$ ratios of less than 1.00. Such an interpretation is not plausible, however, since it then becomes difficult to explain why marine phosphorites from similar areas fail to exhibit similar evidence of secondary addition of uranium. More likely, the formation of phosphorites off Peru has been a more or less continuous process from at least late Pleistocene time to the present; thus, reworking and continued enrichment of older phosphorites would lead to a pre-Recent age bias in some of the nodules.

If phosphorites are currently forming off Peru, as our data indicate, a systematic study of the mode of occurrence of these deposits in relation to pertinent oceanographic parameters should lead to a better understanding of the processes of phosphorite genesis. Knowledge of the distribution pattern of the phosphorites with respect to the boundaries of the oxygen minimum layer where the latter impinges on the continental margin may hold an important clue to the mode of phosphorite formation and should serve as a guideline for future studies in this area.

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Human Chorionic Gonadotropin: Its Possible Role in Maternal Lymphocyte Suppression

Abstract. *Human chorionic gonadotropin completely inhibits the response of lymphocytes to phytohemagglutinin. The effect is both reversible and noncytotoxic. These observations support the theory that the fetus is accepted because human chorionic gonadotropin represents trophoblastic surface antigen and blocks the action of maternal lymphocytes.*

The fact that the mother accepts her fetus as an allograft when a large percentage of the potential antigenic sites of the fetus are different from those of the mother defies precise explanation by transplantation biologists (*1*). At the center of this enigma lies the question of how the fetal trophoblast can successfully engraft itself on the maternal endometrium while it is intimately and constantly exposed to presumably immunocompetent maternal lymphocytes.

Table 1. Effect of HCG on the incorporation of [^{3}H]thymidine by lymphocytes stimulated with PHA in a single individual. Cultures at each concentration of HCG were done in quintuplicate. Control culture contained PHA but no HCG. Background culture contained neither PHA nor HCG. Results are expressed in both counts per minute and a percentage of the control and are per 2×10^5 lymphocytes; S.E.M., standard error of the mean.

HCG (I.U./ml)	[^{3}H]Thymidine incorporation			
	Inhibitory effect of HCG		Reversibility of inhibitory effect of HCG	
	Mean ± S.E.M. (10^3 count/min)	Percent	Mean ± S.E.M. (10^3 count/min)	Percent
Control	19.8 ± 2.8	100	30.2 ± 4.4	100
10	15.3 ± 0.7	77	35.1 ± 2.7	116
100	15.6 ± 1.8	79	32.2 ± 1.6	106
1,000	9.5 ± 1.7	48	34.4 ± 1.0	114
2,500	3.6 ± 0.3	18		
5,000	1.9 ± 0.4	9		
10,000	0.7 ± 0.1	4	34.2 ± 1.3	113
Background	0.17 ± 0.03	0.9	0.07 ± 0.004	0.2

It has been suggested that the trophoblastic antigens are masked by a surface layer of mucoprotein which allows the successful graft-host relationship (*2*). An alternate hypothesis has been that pregnancy alters the immunocompetence of maternal lymphocytes in some manner and thereby permits the implantation and development of the genetically dissimilar fetus. Recent studies have presented conflicting evidence as to whether there may be an alteration in maternal lymphocyte function during pregnancy (*3*) and whether maternal plasma may suppress lymphocyte response to either the plant lectin phytohemagglutinin (PHA) (*3*) or allogenic lymphocytes (*4*).

Human chorionic gonadotropin (HCG) is a glycoprotein which is produced in large amounts by the trophoblast. It is readily detectable in maternal serum in the first 2 weeks of pregnancy (*5*). Some inhibitory effect of HCG on lymphocyte transformation has been demonstrated (*6*). It occurred to one of us (M.A.N.) that the carbohydrate portion of HCG, which is 31.3 percent of the glycoprotein by weight (*7*), may represent trophoblastic cell surface antigen by analogy with the well-known fact that some glycoproteins in the saliva are carriers of the ABO blood group antigens (*8*). If this were the case, then a large concentration of HCG surrounding the engrafting trophoblast at implantation may serve to block the rejection of the trophoblast by maternal lymphocytes. Accordingly, as an initial step in investigating this possibility, we undertook a quantitative study of the effects of HCG on the response of human lymphocytes to PHA. Immunocompetent lymphocytes which are exposed in vitro to PHA will be stimulated to divide. This cell division can be assayed by recording increased uptake of [^{3}H]thymidine by the lymphocyte cell culture.

Blood was collected in heparinized plastic syringes from healthy donors, including adult males and nonpregnant and pregnant females. A leukocyte-rich suspension was prepared with methylcellulose and Hypaque (*9*) and then resuspended in a mixture of tissue culture medium (*10*) which contained 20 percent (by volume) inactivated human AB serum, 40 m*M* Hepes buffer (*10*), and 3 percent (by volume) penicillin-streptomycin-Fungizone solution.

The lymphocyte culture system used in the first four experiments was a modification of that described by Caron *et al.* (*11*). In the remaining eight studies, U-bottomed microtiter plates and the MASH-I (multiple automated sample harvester) were used (*12*). In both methods, 2×10^5 lymphocytes per milliliter were incubated for 1 hour at 37°C with various concentrations of HCG (*10*), which was dissolved in the above tissue culture medium. The concentrations of HCG ranged from 1 to 10,000 international units (I.U.) per milliliter final volume, and the cultures were prepared in either quadruplicate or quintuplicate. Either a 1/400 dilution or a 1/1600 dilution of PHA (*10*) was added, and the cultures were incubated for 3 days at 37°C in a humidified atmosphere of 5 percent CO_2. Controls containing no HCG were prepared in each study. The cells were then incubated for 2 hours in the presence of 2 μc/ml of [^{3}H]thymidine and harvested, and the incorporation of [^{3}H]-thymidine was measured in a liquid scintillation counter (counts per minute per 2×10^5 lymphocytes).

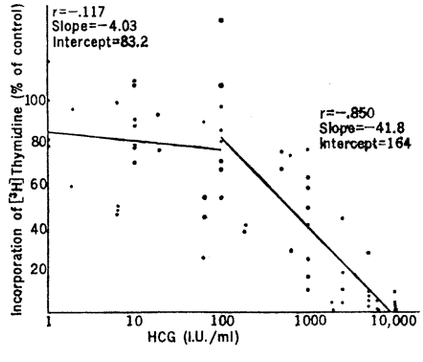

Fig. 1. Pooled data from 12 experiments showing the effect of HCG on human lymphocyte blastogenic response to PHA as measured by [^{3}H]thymidine incorporation. Each data point is expressed as a percentage of that individual's control culture in which no HCG was present. The difference between the control and the mean incorporation over the range of 1 to 100 I.U./ml is significant ($.001 < P < .005$, *t*-test). The slope of the regression line for concentrations from 100 to 10,000 I.U./ml is highly significant ($P < .0005$, *F* ratio test).

HCG inhibits the human lymphocyte response to either dose of PHA (Fig. 1). Each data point represents the percentage of an individual's control culture when no HCG was present during incubation. The control value, therefore, represents 100 percent incorporation of [^{3}H]thymidine by the lymphocytes (Table 1). The data are described as the percentage of [^{3}H]thymidine incorporation plotted against the log of the concentration of HCG (Fig. 1). Two linear regression equations with their correlation coefficients (*r*) further elucidate the pooled data. There is a significant inhibitory effect of even the relatively low concentrations of HCG from 1 to 100 I.U./ml when compared with the controls ($.001 < P < .005$). However, at these low concentrations the effect is not significantly related to the amount of HCG ($r = -.117$, and $.25 < P < .90$). A striking concentration-dependent relationship over the range of 100 to 10,000 I.U./ml is clearly demonstrated ($r = -.849$, and $P < .0005$).

We performed a separate experiment designed to evaluate reversibility of the HCG effect and cell viability after exposure to HCG. The lymphocytes were incubated for 1 hour in various concentrations of HCG, centrifuged, and resuspended in the supplemented medium. When PHA was added and the cells were incubated for 3 days, the incorporation of [^{3}H]thymidine was essentially identical to that of the control (Table 1). Since the HCG could be

washed from the cells, its inhibitory effect was reversible. The trypan blue exclusion technique for quantifying the number of viable cells (*13*) showed that viability of controls and lymphocytes incubated for 72 hours in concentrations as high as 10,000 I.U./ml of HCG was identical.

Our studies indicate that HCG is a potent and reversible inhibitor of the response of human lymphocytes to PHA and that the inhibition occurs without cytotoxicity. HCG in concentrations as little as 1 I.U./ml causes some effect, and the effect becomes marked at and above 100 I.U./ml, which is within the range of the concentration of HCG in human serum during the last half of gestation (*14*). More important, however, is the essentially complete inhibition of the lymphocyte activation achieved by 10,000 I.U./ml. The studies of Braunstein *et al.* (*5*) suggest that trophoblasts from the 10-day embryo could produce local concentrations, where the maternal lymphocytes are in actual contact with the trophoblasts, which far exceed 10,000 I.U./ml. These data suggest that maternal lymphocyte immunocompetence during pregnancy may be altered by HCG in a reversible, noncytotoxic manner. Although PHA stimulation of lymphocytes is a standard test in evaluating lymphocyte function, its relation to physiological and pathological stimulation is unclear. It is necessary to further investigate this inhibitory effect of HCG with the use of other systems to evaluate immunocompetence.

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Synergism of Insecticides by Herbicides

Abstract. *The herbicides atrazine, simazine, monuron, and 2,4-D (2,4-dichlorophenoxyacetic acid) enhanced the toxicity of selected insecticides to* Drosophila melanogaster *Meigen,* Musca domestica *L., and larvae of* Aedes aegypti *L. The insecticides—nine organophosphorus compounds, two chlorinated hydrocarbons, and one carbamate—were used at dosages that resulted in low insect mortalities, while the herbicides by themselves were nontoxic. Atrazine was most effective. With increasing amounts of this herbicide and constant amounts of some insecticides, increasing mortalities of fruit flies were observed. Exposure of the insects for 24 hours to carbofuran (0.5 microgram),* p,p'*-DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] (4 micrograms), parathion (0.35 microgram), and diazinon (0.2 microgram) alone resulted in mortalities of 7.5, 9.5, 8, and 10.5 percent, respectively. Based on dosage mortality curves obtained with increasing amounts of atrazine, mortalities of 50 percent of the insect populations would have been achieved with 23, 40, 6, and 10 micrograms of atrazine added to the above-mentioned dosages of carbofuran, DDT, parathion, and diazinon, respectively.*

The effects of pesticides or other synthetic chemicals on biological systems have usually been investigated by utilizing one particular test chemical. However, under actual environmental conditions, particularly in agricultural situations, a mixture of synthetic chemicals or their metabolites (or both) is present, and these chemicals may interact in biological systems. For example, detergents increase the persistence and toxicity in soils of the organophosphorus insecticides parathion and diazinon (*1*), reduce the penetration of parathion into pea roots, and inhibit the translocation of lindane into pea greens (*2*). Street (*3*) stressed the ecological significance of pesticide interactions, stating that "DDT and dieldrin are additive at low dosages in inducing testosterone metabolism in pigeon liver." Street *et al.* (*4*) compared the effects of polychlorinated biphenyl compounds (PCB's) and organochlorine pesticides on the induction of hepatic microsomal enzymes. Tsao *et al.* (*5*) reported that Aroclor 5460 greatly increased the residual toxicity

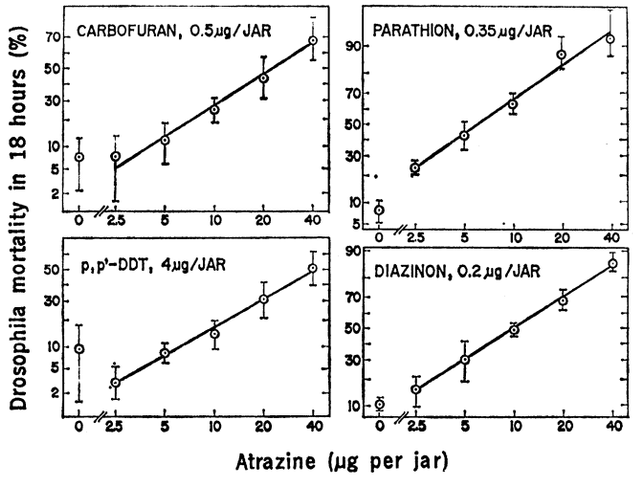

Fig. 1. Effects of increasing dosages of atrazine (0 to 40 µg per jar) in synergizing the toxicities of insecticides applied at constant dosages with the herbicide.