The average mean annual temperature on San Miguel Island (located ~ 15 km northwest of Santa Rosa) is 13.7°C, compared with 16.1°C at the general location (Newport Beach) where the Laguna skull was found (*19*). With an Arrhenius activation energy (E_a) of 33.4 kcal mole^{-1} (*9*), the calculated value for k_{asp} at 13.7°C is 6.6×10^{-6} yr^{-1}. Substituting this value and the measured D/L aspartic acid ratio into Eq. 1 gives an age of 33,000 years, which is in good agreement with the radiocarbon ages deduced for the mammoth skeleton. We feel that this correlation provides additional strong evidence that the ages deduced for the California Paleo-Indian samples are accurate.

The ages given in Table 2 are the oldest direct dates determined so far for any New World hominids. They suggest that man had populated the New World substantially earlier than ~15,000 to ~25,000 years B.P., the last time the Bering Sea land bridge existed (*20*, *21*). Sea level was lower at earlier times (*20*, *21*), with a level low enough for the land bridge formation to occur perhaps ~70,000 years B.P. (*21*). It would appear, based on the limited number of dates we have obtained, that man might have migrated to the New World during this time.

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22. The samples from San Diego were provided by S. Rogers and J. Moriarty, those from Los Angeles by C. Rozaire and R. Berger, and the Santa Rosa Island mammoth bone was provided by R. Berger and P. Orr; we express our gratitude to these individuals for providing the samples. We thank Linda and J.C.W. for encouragement. This work was supported by NSF grant GA-40804. R.A.S. is a Scripps Industrial Associates predoctoral fellow.

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Radioreceptor Assay of Human Chorionic Gonadotropin: Detection of Early Pregnancy

Abstract. *A rapid, sensitive, and specific radioreceptor assay for the determination of human chorionic gonadotropin and luteinizing hormone in plasma is described. Plasma membranes of bovine corpora lutea of early pregnancy, which bind biologically active labeled human chorionic gonadotropin, have been used as receptor. Pregnancy could be detected by assaying the gonadotropin in plasma samples obtained from day 6 to 8 after conception.*

It is well established that the target tissue receptors bind biologically active molecules and that hormone-receptor interaction is of high affinity and specificity (*1*). The specific receptors in target tissues have permitted the measurements of hormones in biological fluids in radioreceptor assays in vitro based on the principle of competitive protein binding methods (*2*). The plasma membranes of bovine corpus luteum exhibit a specific binding ability for bovine LH (*3*, *4*), human LH and hCG (*5*). The receptor is specific for hCG and LH without apparent species specificity; FSH, TSH, hGH, and PRL (*3*) do not bind to the hCG-LH receptor. We now report a radioreceptor assay for quantitative determination of hCG and LH in plasma, with the use of the plasma membrane of bovine corpus luteum of pregnancy as receptor. The assay procedure can be used in the detection of pregnancy in humans as early as 6 days after conception.

Fresh bovine corpora lutea were homogenized in chilled 0.01*M* tris-HCl buffer, *p*H 7.8, containing 1 m*M* $MgCl_2$ and 1 m*M* dithiothreitol and centrifuged at 480*g* for 10 minutes (Sorvall refrigerated centrifuge). The supernatant was again centrifuged at 10,000*g*, and the pellet was purified by zonal centrifugation in sucrose density gradient (*6*) (Beckman ultracentrifuge, model L3-50) to obtain plasma membranes in high yield. Highly purified hCG, containing 12,000 international units per milligram (*7*), was labeled with ^{125}I (Cambridge Nuclear, Cambridge, Mass.), as follows (*8*). A solution of 2 mc of ^{125}I in 20 μl of 0.1*M* sodium acetate buffer, *p*H 6.0, was mixed with 25 μg of hCG. Lactoperoxidase (Sigma RZ = 0.78) (50 ng in 20 μl of buffer) and 200 ng of H_2O_2 in 10 μl of water were added. Then three 100-ng portions of H_2O_2 were added at 5-minute intervals. At the end of 20 minutes the reaction was stopped by the addition of 0.5 ml of 0.15*M* NaCl containing 1 percent bovine serum albumin (BSA), *p*H 7.0. The labeled hormone was then separated from free iodine by gel filtration on a column (1 by 30 cm) of Sephadex G-100 equilibrated with 0.15*M* NaCl containing 1 percent BSA, *p*H 7.0. The labeled hCG in the unretarded fraction showed specific activity in the range of 40 to 50 μc/μg and a biological activity of 8923 I.U./mg (95 percent confidence limits of 5,826 to 12,250 I.U.) (*5*).

Our subjects were individuals who

came to the fertility clinic because of failure to achieve conception. On the basis of their daily basal body temperatures, and daily excretions of estrogen and progesterone in the urine as well as characteristic changes of the cervical mucus, the time of ovulation was approximated, and the patients were accordingly advised to conceive. Blood was collected from them daily, following last menstrual period, throughout the cycle. The day of ovulation was subsequently confirmed by the LH surge. Four women who became pregnant were the source of the data for our study.

All blood samples were collected in heparinized tubes. Blood samples were also obtained from women during postpartum lactation; from patients with acromegaly and hypothyroidism, and from normal men. Blood samples were also examined from four normal women throughout the complete menstrual cycle of each. The blood was centrifuged and plasma samples were stored at − 20°C until used in the assay.

The procedure for the radioreceptor assay was as follows. All dilutions were made in 10 m*M* tris-HCl buffer of *p*H 7.2 containing 0.1 percent BSA, 1 m*M* $CaCl_2$; 20 I.U. of Trasylol (an enzyme inhibitor; FAB Pharmaceuticals) in 100 μl of tris-HCl buffer. For standard curve each (75 by 100 mm glass disposable) tube contained 100 μl of hCG solution (12,000 I.U./mg) at the following concentrations: 3.0, 6.2, 12.5, 25.0, 50.0, and 100 ng/ml. For unknown samples 100 μl of a 1 : 2 to 1:50 dilution of plasma was added. To each tube was added ^{125}I-labeled hCG (1.5 ng; ≃ 50,000 count/min), and 40 μg of plasma membrane (protein) in 100 μl of tris-HCl buffer. The standards and plasma samples were incubated at 37°C for 20 minutes (*5*). After incubation the tubes were placed in an ice bath, and 1 ml of chilled tris-HCl buffer was added to each tube. The contents of the tubes were mixed with a Vortex mixer and centrifuged for 10 minutes at 5000*g*. The supernatants were aspirated, and the radioactivity in the pellets was counted in an Autogamma counter (Packard Instruments).

The logit-log transformation of the dose response curve indicated that the sensitivity of the method was greater than 3 ng/ml. The minimum quantity of hCG which could be detected in the assay was 50 to 75 pg with a precision of ± 15 percent. A dose response obtained with various dilutions of plasma from a pregnant subject yielded a slope similar to that of hCG, indicating the validity of the assay. There was no cross-reaction by plasma from acromegalic subjects with high hGH levels, by plasma from subjects with hypothyroidism with high levels of TSH, by plasma from subjects during postpartum lactation (which contained high levels of PRL), or by purified human PRL, indicating the specificity of hCG-LH measurements. The concentration of hCG-LH in the plasma samples during luteal phase as well as in the plasma samples obtained after conception are presented in Fig. 2. The values during the luteal phase are below 3 ng/ml, whereas the values are 2- to 20-fold higher in the plasma samples of pregnant women. The levels of LH in the plasma of normal male subjects were within the limits of detection. The levels of hCG in pregnant subjects increased progressively with advancing pregnancy, and on days 18 to 20 hCG levels were more than 100 ng/ml. It may be emphasized that the levels of luteal phase were similar to those measured by radioimmunoassay (*9*). Previous data have shown that hCG and LH bind to the same receptor (*4, 5*), and therefore the hormone levels obtained during the luteal phase should represent only LH. In the case of pregnant women the levels obtained may be due to hCG or LH, or gonadotropin-like pregnancy specific substance. The absolute hCG-LH levels during pregnancy, were, however, significantly higher than those during the luteal phase. The delayed LH surge could be ruled out by performing the test on two or more consecutive days. Detection of pregnancy in menopausal subjects with high levels of LH may, however, require additional confirmatory tests (*12*).

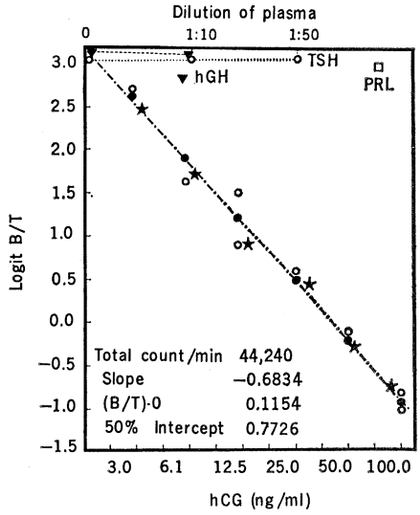

Fig. 1. Computer output of logit-log dose-response curve for the radioreceptor assay of hCG-LH; *hGH*, plasma from acromegalic subjects; *TSH*, plasma from hypothyroid patients; *PRL*, plasma from women with post-partum lactation.

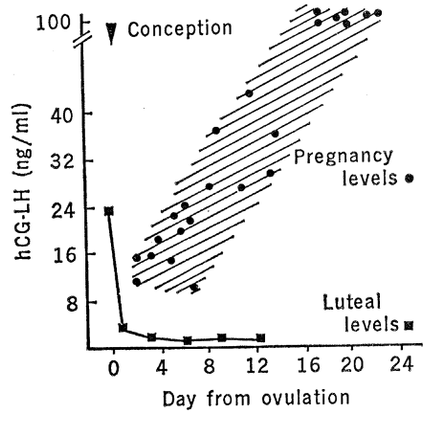

Fig. 2. Levels of hCG and LH during the luteal phase of the menstrual cycle and during early pregnancy in four subjects. Day 0 represents the day of the midcycle LH surge.

The source of hCG-LH in the blood as early as day 4 to 6 is still unknown. The gonadotropin-like substance, similar to hCG or LH, appears to be specific for early pregnancy and may be secreted by the blastocyst; or a signal from the fertilized blastocyst may sustain high levels of pituitary LH in early pregnancy until the placental secretion of hCG is established. These hypotheses remain to be substantiated by direct experimental evidence. We have detected a gonadotropin similar to hCG-LH in rabbit blastocysts prior to implantation (*10*). Biologically inactive molecules such as desialylated hCG bind to the receptor in vitro (*2*); however, such a molecule, if present, should not sustain pregnancy. Hence the possibility of a false positive result is very little. This has now been documented by the detection of early pregnancy in more than 80 cases without error. However, the possibility of an error cannot be ruled out in the absolute sense. Bioassays are insensitive, require large amounts of body fluids, and fail to provide quantitative information (*11*). The radioimmunossays of LH and hCG are sensitive and have been used in the detection of pregnancy as early as day 9 (*12*). Use of bioassay and immunological techniques may

sometimes yield false results especially during early pregnancy (*13*). The radioreceptor assay reported here is rapid, specific, and sensitive enough to detect pregnancy as early as day 6 to 8 after conception, and is of use in the diagnosis and clinical management of early pregnancy, abortion, ectopic pregnancy, infertility, related pathophysiological conditions, and in the evaluation of suction curettage and artificial insemination (*14*).

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Serum Induced Lymphoid Cell Mediated Cytotoxicity to Human Transitional Cell Carcinomas of the Genitourinary Tract

Abstract. *In some serums of patients with transitional cell carcinoma (TCC), a factor is present which induces lymphocytes from most donors with or without TCC to become cytotoxic against TCC-derived target cells. The induced cytotoxicity was directed against target cells derived from TCC's of the renal pelvis, ureter, and urinary bladder, but not against cells derived from normal kidney, bladder, testis, or skin or from renal cell carcinoma. Cytotoxicity occurred without complement but did not occur without effector cells.*

In animals, tumor-specific, lymphocyte mediated cytotoxicity (LMC) has been demonstrated by means of assays in vitro (*1*). This in vitro LMC can be abrogated by the addition of serum factors present in so-called blocking serums (*1*). In contrast to the reduction in LMC caused by blocking serums, it has been demonstrated that certain immune serums contain other factors (lymphocyte dependent antibodies) which induce tumor-specific cytotoxicity by lymphocytes from immune and nonimmune donors (*2*). This lymphocyte dependent antibody (LDA) is an immunoglobulin G (IgG) which does not

Table 1. Target cell survival after exposure to LDA-like activity of serums from patients T5 and T6 with and without effector cells (lymphocytes). Serum donors T5 and T6 were diagnosed as having transitional cell carcinoma of the bladder; donors C1 and C3 were normal. All serums were diluted 1 : 5 in phosphate buffered saline, except as indicated; S.E.M., standard error of mean; CI, cytotoxic index; ns, not significant.

Lymphocyte donor (diagnosis)	Serum donor	Surviving cells			
		Type	Number ± S.E.M.	CI	*P*
		Experiment 1			
None	C3	TCC-bladder	78.0 ± 9.0		
None	T6		93.5 ± 9.3	−20	ns
T1 (TCC-pelvis)	C3		76.2 ± 8.5		
T1	T6		15.0 ± 1.8	80	.01
None	C3(1 : 20)		74.3 ± 9.4		
None	T6(1 : 20)		68.3 ± 8.6	8	ns
T1	C3(1 : 20)		79.5 ± 2.1		
T1	T6(1 : 20)		49.2 ± 6.6	38	.01
		Experiment 2			
None	C3	TCC-pelvis	52.2 ± 1.6		
None	T5		46.7 ± 3.0	11	ns
T1 (TCC-pelvis)	C3		28.2 ± 4.4		
T1	T5		13.2 ± 1.1	53	.01
C1 (normal)	C3		29.3 ± 1.5		
C1	T5		28.0 ± 1.6	5	ns
T2 (TCC-bladder)	C3		17.2 ± 1.5		
T2	T5		10.2 ± 1.0	41	.01
T3 (TCC-bladder)	C3		35.2 ± 1.9		
T3	T5		29.0 ± 1.8	18	.05
		Experiment 3			
None	C1	TCC-bladder	52.5 ± 3.6		
None	T5		64.8 ± 19.0	−23	ns
T1 (TCC-pelvis)	C1		18.0 ± 1.8		
T1	T5		0.8 ± 0.2	96	.01
T2 (TCC-bladder)	C1		51.4 ± 5.4		
T2	T5		0.5 ± 0.3	99	.01
T4 (TCC-bladder)	C1		36.5 ± 6.7		
T4	T5		1.0 ± 0.6	97	.01
C2 (normal)	C1		63.8 ± 7.5		
C2	T5		15.6 ± 2.3	76	.01
None	C1	Normal kidney	73.3 ± 8.8	− 5	ns
None	T5		77.0 ± 3.0		
T1 (TCC-pelvis)	C1		44.6 ± 7.0	− 4	ns
T1	T5		46.4 ± 4.2		
T2 (TCC-bladder)	C1		57.0 ± 6.0	2	ns
T2	T5		56.0 ± 4.8		
T4 (TCC-bladder)	C1		52.0 ± 3.6	12	ns
T4	T5		45.8 ± 2.7		
C2 (normal)	C1		47.3 ± 2.4	− 3	ns
C2	T5		48.7 ± 2.6		