lated synthesized autoantibodies that react with cells in the anterior pituitary. Some react with GH-producing cells, and others react with different but still unidentified subpopulations of cells. The differences in the species specificities of the monoclonal autoantibodies directed against GH suggests that these antibodies recognize different antigenic determinants on the GH molecule. Although human and rat GH are serologically different (*9*), the fact that at least one of the monoclonal antibodies (that is, 5B-5) reacts with GH from both species suggests that this antibody recognizes a shared antigenic determinant. The different reactivity patterns of these autoantibodies also suggest that they may serve as useful probes in the fine analysis of antigenic and evolutionary relatedness of hormones.

The availability of large quantities of purified autoantibodies should make it possible to address a number of fundamental questions concerning autoimmunity. First, it should be possible to determine whether different individuals with the same disease develop autoantibodies against the same molecules, and if so, whether the autoantibodies are directed against the same antigenic determinants. Second, these monoclonal autoantibodies can be used in affinity columns to isolate and identify some of the still unknown autoantigens. Third, these monoclonal autoantibodies should aid in determining, by passive transfer experiments, what role autoantibodies actually play in pathogenesis. Although the present study involves murine autoantibodies triggered by a viral infection, it should be possible to obtain and study monoclonal autoantibodies from human polyendocrine disease.

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References and Notes

1. N. Talal, in *Clinical Immunology*, C. W. Parker, Ed. (Saunders, Philadelphia, 1980), p. 86.
2. D. Glass and P. H. Schur, in *Autoimmunity: Genetic, Immunologic, Virologic and Clinical Aspects*, N. Talal, Ed. (Academic Press, New York, 1977), p. 532.
3. W. J. Irvine, B. F. Clarke, L. Scarth, D. R. Cullen, L. J. P. Duncan, *Lancet* **1970-II**, 163 (1970); A. C. MacCuish, E. W. Barnes, W. J. Irvine, L. J. P. Duncan, *ibid.* **1974-II**, 1529 (1974); G. F. Bottazzo and R. Lendrum, *ibid.* **1976-II**, 873 (1976); R. Lendrum, J. G. Walker, A. G. Cudworth, C. Theophanides, D. A. Pyke, A. Bloom, D. R. Gamble, *ibid.*, p. 1273; A. Lernmark, Z. R. Freedman, C. Hofmann, A. H. Rubenstein, D. F. Steiner, R. L. Jackson, R. J. Winter, H. S. Traisman, *N. Engl. J. Med.* **299**, 375 (1978); M. J. Dobersen, J. E. Scharff, F. Ginsberg-Fellner, A. L. Notkins, *ibid.* **303**, 1493 (1980); S. Serjeanton, J. Theophilus, P. Zimmet, J. Court, J. R. Crossley, R. B. Elliott, *Diabetes* **30**, 26 (1981); S.-W. Huang, L. H. Haedt, S. Rich, J. Barbosa, *ibid.*, p. 873; R. Mirakian, A. G. Cudworth, G. F. Bottazzo, C. A. Richardson, D. Doniach, *Lancet* **1982-I**, 755 (1982); S. Baekkeskov, J. H. Nielsen, B. Marner, T. Bilde, J. Ludvigsson, A. Lernmark, *Nature (London)* **298**, 167 (1982).
4. V. A. Lenon, in *The Menarini Series on Immunopathology*, P. A. Miescher, Ed. (Springer-Verlag, New York, 1978), vol. 1, p. 178; B. G. W. Arnason, in *Clinical Immunology*, C. W. Parker, Ed. (Saunders, Philadelphia, 1980), p. 1088.
5. T. Onodera, A. Toniolo, U. R. Ray, A. B. Jenson, R. A. Knazel, A. L. Notkins, *J. Exp. Med.* **153**, 1457 (1981).
6. T. Onodera, U. R. Ray, K. A. Melez, H. Suzuki, A. Toniolo, A. L. Notkins, *Nature (London)* **297**, 66 (1982).
7. H. Koprowski, W. Gerhard, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2985 (1977).
8. T. J. McKearn, in *Monoclonal Antibodies Hybridomas: A New Dimension in Biological Analysis*, R. H. Kennett, T. J. McKearn, K. B. Bechtol, Eds. (Plenum, New York, 1980), p. 374.
9. T. Hayashida, in *Hormonal Proteins and Peptides*, C. H. Li, Ed. (Academic Press, New York, 1975), vol. 3, p. 41.

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Biologically Active Chorionic Gonadotropin: Synthesis by the Human Fetus

Abstract. *The kidney, and to a slight extent the liver, of human fetuses were found to synthesize and secrete the α subunit common to glycoprotein hormones. Fetal lung and muscle did not synthesize this protein. Since fetal kidney and liver were previously found to synthesize β chorionic gonadotropin, their ability to synthesize bioactive chorionic gonadotropin was also determined. The newly synthesized hormone bound to mouse Leydig cells and elicited a biological response: namely, the synthesis of testosterone. These results suggest that the human fetus may participate in metabolic homeostasis during its development.*

The concept that hormonal peptides are a unique product of a single cell type contained within a specialized organ known as an endocrine gland has proved to be too restrictive. Cells producing certain hormones occur outside these glands, and malignancies of nonendocrine tissues elaborate peptide hormones (*1*). Other cell types (nonendocrine and nonmalignant) also produce hormonal peptides (*1–4*). Recent studies on the human fetal-placental unit show that the placenta is a source of endorphin, adrenocorticotropin (ACTH), and hypothalamic releasing hormones, and that the fetal lung may produce ACTH and bombesin (*5*). Investigators in our laboratory have shown that large amounts of immunoreactive human chorionic gonadotropin (hCG) occur in a variety of human fetal tissues (*6*) and that synthesis of the β subunit of this hormone can occur in

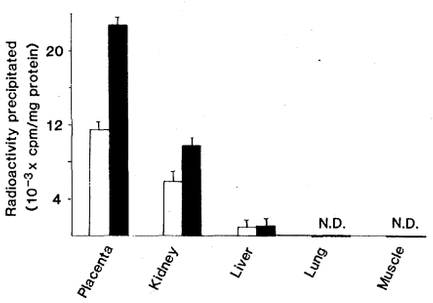

Fig. 1. Synthesis of α subunit by human fetal tissue and its characterization. Immunoprecipitation of newly synthesized α subunit. Placenta and fetal kidney, liver, lung, and muscle were obtained in the fresh state. The gestations were uncomplicated and ranged from 16 to 19 weeks. Approximately 1 g of each tissue was cut into 2-mm slices and placed in 10 ml of Krebs-Ringer bicarbonate buffer, *p*H 7.4, containing 0.2 percent glucose. The slices were incubated for 30 minutes at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2, after which time the medium was changed and 20 μCi of [^{35}S]methionine was added. After incubation for another 12 hours the medium and tissues were separated. The tissues were homogenized in 0.15*M* NaCl, 5m*M* EDTA, and 0.1*M* tris containing 0.1 percent Triton X-100 (NET-TX). Samples were centrifuged at 40,000*g* for 1 hour at 40°C. The supernatants were decanted and 5 μl of a 10 percent suspension of *Staphylococcus aureus* capsule (SAC) was added per milliliter of supernatant collected. The centrifugation was then repeated. The α subunit was precipitated by mixing 100 μl of the tissue homogenates or incubation media with 50 μl of antiserum diluted 1:250 and allowing the mixture to react overnight at 4°C. Ten microliters of SAC were added and allowed to react for 15 minutes at room temperature and sedimented by centrifugation at 12,000*g* for 1 minute. The complexes were washed three times with 100 μl of NET-TX buffer to remove nonspecifically bound radioactivity. After the final wash, the SAC complex was disrupted by exposing it to 0.1*N* KOH for 30 minutes. The supernatant was decanted and counted in a liquid scintillation counter (PRIAS, Packard Instruments). The radioactivity is expressed per milligram of protein as determined by a micro-Bradford assay. The results represent triplicate determinations on six separate fetuses ± standard error of the mean for media (solid bars) and tissue homogenates (open bars).

fetal kidney and, to a slight extent, in fetal liver (*7*). We now report that fetal tissues can also synthesize the α subunit common to glycoprotein hormones, and that the newly synthesized hormone is bioactive. These observations constitute evidence that a nonendocrine fetal tissue can synthesize a bioactive peptide that is immunologically identical to hCG, and support the hypothesis that the fetus may participate in metabolic homeostasis during its development.

High concentrations of hCG were found previously in extracts of fetal thymus, gonad, and kidney (*6*). Since thymus and gonad cannot be reliably identified in abortion material, we chose to investigate whether the observed high renal concentration of hCG resulted from sequestration of circulating hormone or the de novo synthesis of hCG by this organ. Tissues were identified from 16- to 19-week fetuses immediately after they were aborted by dilatation-evacuation during uncomplicated pregnancies. All fetal tissues were examined microscopically and found to be free of contaminating trophoblastic tissue before use. Specimens of placenta, kidney, liver, lung, and muscle were minced separately and placed in Krebs-Ringer bicarbonate buffer containing 0.2 percent glucose. After 30 minutes of incubation the buffer was replaced and [^{35}S]methionine was added. After a further 12 hours of incubation at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2, the tissues and medium were separated. Tissue samples were homogenized in a buffer containing 0.1 percent Triton X-100, with the medium being brought to the same concentration of the detergent. All samples were then centrifuged for 1 hour at 40,000*g*. A suspension of attenuated *Staphylococcus aureus* cells (SAC) was added and the centrifugation repeated; this procedure reduced nonspecific binding to the capsule during subsequent immunoprecipitation.

The α subunit was precipitated with a rabbit antiserum to intact hCG from which β-hCG determinants had been absorbed by affinity chromatography. The affinity column was prepared by linking 1 to 2 mg of β-hCG (CR123 β, NIAMMD) to 3 g of Affi-gel 10 (Bio-Rad) essentially as recommended by the manufacturer. After unreacted sites were blocked with leucine methyl ester and extensive washing of the gel, the resin was packed in a syringe and 2 to 3 ml of antiserum were placed in the column and left there for 2 to 3 hours. The subsequent drop-through fraction was found to be free of antibodies to β-hCG by means of a binding assay with ^{125}I-labeled β-hCG.

The absorbed antiserum was added to portions of tissue homogenates and incubation media. Immune complexes were precipitated with 10 percent SAC, and were washed three times in buffer containing 0.1 percent Triton X-100. The complex was dissociated with base and the precipitated protein counted in a liquid scintillation counter. A standard micro-Bradford assay was used to determine protein concentrations. Figure 1 shows the incorporation of [^{35}S]methionine into the immunoprecipitable α subunit per milligram of total protein. Both the placenta and fetal kidney actively synthesized and secreted immunoreactive α subunit. Low but consistent rates of α subunit synthesis were observed in the fetal liver. Immunoreactive protein could not be detected in the fetal lung or muscle. Immunocytochemical studies of fetal tissue show (*8*) that the degree of staining for the α subunit (as well as for β-hCG) closely parallels the observed rate of synthesis of the proteins, with placenta staining more darkly than kidney. Small numbers of hepatocytes appear to have intracellular stores of the subunits, whereas no specific staining occurs in the lung or muscle (*8*).

To ensure that only the α subunit was being recognized by the precipitation procedure, we subjected the precipitated protein, after its dissociation from the

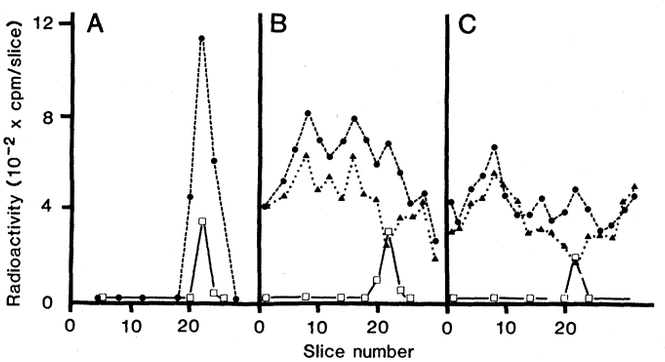

Fig. 2. Electrophoretic analysis of immunoprecipitated protein. Tissue homogenates were concentrated tenfold by means of Amicon filters and then precipitated. After precipitation the SAC complex was disrupted by exposure to SDS buffer (0.01*M* sodium phosphate, *p*H 7.0, 1 percent SDS, 1 m*M* β-mercaptoethanol) and boiling for 1 minute. After the SAC had been sedimented, the samples were subjected to electrophoresis on SDS disk-gels according to a modification of the Weber Osborne method with the use of a 10 percent acrylamide running gel (0.5 by 8 cm) and a 4 percent acrylamide stacking gel (0.5 by 1 cm). The gels were run at *p*H 8.6 and 6 mA per gel until the bromphenol blue dye front neared the bottom of the gel. Gels were frozen on dry ice and cut into 2-mm slices. The slices were placed in scintillation vials containing 3 percent Protosol in Scintiverse (Fisher Scientific), heated to 60°C overnight, and counted. (A) Electrophoresis of ^{125}I-labeled α subunit before (●) and after (▲) immunoprecipitation. The subunit has an R_F of 0.82 and comigrates with highly purified α subunit. No radioactivity was detected after precipitation, but the subunit was noted after the SAC complex had been disrupted and the precipitated protein electrophoresed (□). (B and C) Analysis of (B) placental and (C) kidney tissue extracts before (●) and after (▲) precipitation. Electrophoresis of the precipitated protein resulted in a single band that comigrated with purified α subunit (□). No changes occurred in the electrophoretic patterns of liver, lung, or muscle extracts before or after precipitation.

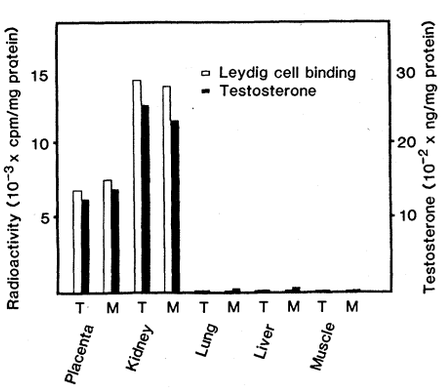

Fig. 3. Mouse Leydig cell bioassay for human luteinizing hormone (hLH) and hCG activity. Cells from the testes of 5- to 7-week-old mice were mechanically dispersed in Medium 199 containing Hanks salts, glutamine, collagenase, and deoxyribonuclease. After dispersion, the cell concentration was adjusted to 400,000 Leydig cells per 100 μl of Medium 199 containing Hanks salts. Human LH and hCG activity was measured by radioimmunoassay of testosterone biosynthesis after 3 hours of incubation with standard hCG (0 to 80 pg) or 5 μl of tissue homogenate and incubation medium. Any testosterone present in the added medium or homogenate was subtracted from the experimental values. The cells were centrifuged after incubation and before the testosterone assay. The cells were washed extensively in phosphate-buffered saline containing 1 percent gelatin, then solubilized and counted in scintillation fluid containing 3 percent Protosol (Fisher Scientific). The radioactivity bound to the cells was completely removed by the addition of 1000 pg of unlabeled hCG. The radioactivity bound to the Leydig cells and the nanograms of testosterone produced are expressed per milligram of protein added to the cultured cell. *T*, tissue; *M*, medium.

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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References and Notes

1. W. D. Odell and A. R. Wolfson, *Am. J. Med.* **63**, 317 (1980).
2. G. D. Braunstein, J. Rasor, M. E. Wade, *N. Engl. J. Med.* **293**, 339 (1975).
3. J. G. Ratcliffe, R. A. Knight, G. M. Besser, J. Landon, A. G. Stansfield, *Clin. Endocrinol. (Oxford)* **1**, 27 (1972).
4. K. Pavelic, A. Ferle-Vidovic, M. Osmak, S. Vur-Pavlovic, *J. Natl. Cancer Inst.* **67**, 687 (1981).
5. J. Roth, D. LeRoth, J. Shiloach, J. Rosenzweig, M. A. Lesniak, J. Havrankova, *N. Engl. J. Med.* **306**, 523 (1982).
6. I. H. Huhtaniemi, C. C. Korenbrot, R. B. Jaffe, *J. Clin. Endocrinol. Metab.* **46**, 994 (1978).
7. W. G. McGregor, W. J. Raymoure, R. W. Kuhn, R. B. Jaffe, *J. Clin. Invest.* **68**, 306 (1981).
8. P. C. Goldsmith, W. G. McGregor, W. J. Raymore, R. W. Kuhn, R. B. Jaffe, in preparation.
9. W. R. Magle and J. Ramachandran, *Endocrinology* **93**, 127 (1973).
10. M. Seron-Ferre, C. C. Lawrence, R. B. Jaffe, *J. Clin. Endocrinol. Metab.* **46**, 834 (1978).
11. R. B. Jaffe *et al.*, *J. Steroid Biochem.* **8**, 479 (1977).
12. E. S. Gray and D. R. Abramovich, *Am. J. Obstet. Gynecol.* **137**, 491 (1980).
13. I. H. Porter, Ed., *Human and Embryonic Fetal Death* (Academic Press, New York, 1980).
14. W. G. McGregor and R. B. Jaffe, unpublished observations.
15. I. H. Huhtaniemi, C. C. Korenbrot, R. B. Jaffe, *J. Clin. Endocrinol. Metab.* **44**, 963 (1977).
16. We thank H. Papkoff for providing highly purified α subunit. Supported in part by NIH grant HD08478 and a grant from the Cowell Foundation.

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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