de-Thé, G. & Payne, L. N.) 437–444 (International Agency for Cancer Research, Lyon, 1975).
11 Boyd, A. L. & Orme, T. W. *Int. J. Cancer* **16**, 526–538 (1975).
12 Darai, G. & Munk, K. *Int. J. Cancer* **18**, 469–481 (1976).
13 Puck, T. T. *J. Cell. comp. Physiol.* **52**, 287–311 (1958).
14 Russell, W. C. *J. exp. Med.* **102**, 595–601 (1955).
15 Ham, R. G. *Proc. natn. Acad. Sci. U.S.A.* **53**, 288–293 (1965).
16 McDougall, J. K. *Nature* **225**, 456–458 (1970).
17 McPherson, I. & Montagnier, L. *Virology* **23**, 291–294 (1964).
18 Temin, H. M. & Mizutani, S. *Nature* **226**, 1211–1213 (1970).
19 Baltimore, D. *Nature* **226**, 1209–1211 (1970).
20 Klement, V., Rowe, W. P., Hartley, J. W. & Pugh, W. E. *Proc. natn. Acad. Sci. U.S.A.* **63**, 753–758 (1969).

Secretion of hCG-α subunit and hCG by HeLa strains

In recent letters[1,2], we have independently reported seemingly different secretion patterns of human chorionic gonadotropin (hCG) and its alpha (hCG-α) and beta (hCG-β) subunits by certain HeLa strains. These letters were further discussed in an article in News and Views[3]. Ghosh and Cox[1] found that HeLa 65 and HeLa 71 secreted a product with activity in an 'hCG-β' immunoassay (detecting hCG and/or hCG-β), and that this secretion was greatly stimulated by butyrate; hCG-α was not measured. Lieblich *et al.*[2] reported that HeLa CCL 2, 2.1 and 2.2 secreted very large quantities of hCG-α but no detectable amounts of hCG or hCG-β (<0.2 ng ml^{-1}). Two questions were raised: what was the nature of the immunoactive material in the hCG-β assay, and could the differences between the two laboratories be attributed to the known variability among HeLa strains? We have collaborated in an attempt to answer these questions, and we confirmed strain differences in hCG-α secretion in HeLa cells.

HeLa 65 and HeLa 71 were grown in increasing concentrations of sodium butyrate at New York University, and the media were analysed for hCG, hCG-β and hCG-α at the National Institutes of Health. In separate, specific radioimmunoassays (RIA), we have identified the 'hCG-β' immunoactivity as complete hCG; no free hCG-β was found (<1 ng ml^{-1}). Both HeLa strains secreted much larger quantities of free hCG-α than complete hCG (Table 1), but not sufficient hCG-α to produce significant cross reaction in the hCG RIA. Sodium butyrate stimulated both HeLa strains to increase markedly the secretion of both proteins, with the secretion of hCG-α far in excess of hCG. Although the relative stimulation by butyrate of hCG and hCG-α were within an order of magnitude ($\sim$100–500-fold), the absolute levels of hCG-α secretion exceeded complete hCG by 15-fold in HeLa 65 and 500-fold in HeLa 71. These differences between HeLa 65 and HeLa 71 in basal and butyrate-stimulated secretion of hCG and hCG-α, and the previously reported[2] differences among HeLa 2, 2.1 and 2.2 in basal hCG-α secretion may be functional counterparts of the well-described karyotypic differences among strains of HeLa.

Table 1 Basal and butyrate-stimulated secretion* of hCG-α subunit and hCG in two HeLa strains

	Butyrate concentration (mM)	Cell no. ($\times 10^{-6}$)	hCG (pmol per 10^6 cells per day)	hCG-α (pmol per 10^6 cells per day)
HeLa 65	0	17	0.01	<0.03
	1	11	0.6	2.0
	5	2.2	1.1	16
HeLa 71	0	22	0.02	15
	1	8.2	0.4	430
	5	0.3	3.0	1,450

*HeLa cells grown for 3 days in Waymouth's medium containing 10% foetal calf serum with additions of sodium butyrate as shown.

We have tested butyrate stimulation of another cell line producing α subunit ectopically. Preliminary studies in ChaGo (ref. 4), derived from a primary carcinoma of the lung, also show significant butyrate stimulation of hCG-α secretion rates. Demonstration of butyrate stimulation in a second ectopic protein-producing cell line underlines the potential of this reagent in the study of biosynthetic mechanisms.

Jeffrey M. Lieblich
Bruce D. Weintraub
Saul W. Rosen

Clinical Endocrinology Branch,
National Institute of Arthritis,
Metabolism and Digestive Diseases,
Bethesda, Maryland 20014

Nimai K. Ghosh
Rody P. Cox

Division of Human Genetics
Departments of Medicine and Pharmacology,
New York University Medical Center,
New York, New York 10016

Received November 1; accepted December 21, 1976.

1 Ghosh, N. K. & Cox, R. P. *Nature*, **259**, 416–417 (1976).
2 Lieblich, J. M., Weintraub, B. D., Rosen, S. W., Chou, J. Y. & Robinson, J. C. *Nature* **260**, 530–532 (1976).
3 Ectopic Production of HCG Subunits, *Nature* **260**, 480–481 (1976).
4 Lieblich, J. M. *et al. J. natn. Cancer Inst.* **56**, 911–917 (1976).

Effects of immunisation against luteinising hormone releasing hormone on reproduction of the marmoset monkey *Callithrix jacchus*

Luteinising hormone releasing hormone (LHRH) is a decapeptide secreted by the hypothalamus and transported through the hypophysial portal system to the anterior pituitary gland where it acts to stimulate gonadotrophin release[1]. Selective neutralisation of this releasing factor by specific antibodies has been achieved in rodents[2–4], but there has been no report of similar work on primates. Immunisation against hypothalamic releasing hormones is a potentially suitable method for selective control of the secretion of anterior pituitary hormones. In particular, interference with LHRH release might make possible a new approach to contraception. We describe here how antibodies to LHRH were raised in the marmoset, *Callithrix jacchus*, and outline the subsequent effects on gonadal activity and pituitary function.

Ten adult marmosets, five male and five female, were immunised with synthetic LHRH conjugated to bovine serum albumin (BSA) using the carbodiimide technique[5]. Each animal received eight intradermal injections of the conjugate emulsified in Freund's adjuvant (FCA), followed by *Bordetella pertussis* vaccine as an additional adjuvant. Four further animals, two male and two female, were injected with BSA alone in FCA, together with *B. pertussis* vaccine, and served as controls. Each animal received a booster injection approximately 10 weeks after primary immunisation.

Heparinised blood samples were collected once a week from the femoral vein, centrifuged immediately and plasma was stored at -20 °C for subsequent determination of hormone and antibody levels. Testicular and uterine dimensions were also measured once weekly, using calipers. Progesterone, LH, and testosterone were measured by radioimmunoassay techniques described elsewhere[6–8]. The presence of antibody to LHRH was assessed by measuring the percentage binding of approximately 10 pg radioiodinated LHRH by plasma at a dilution of 1 : 500.