control levels by insulin treatment (*28*).

The vitamin D endocrine system has a wide variety of physiologically relevant interactions with other hormones besides insulin; these include parathyroid hormone, calcitonin, growth hormone, prolactin, glucocorticoids, and estrogens (*1*). Our results documenting a vitamin D-pancreas interaction are not surprising in view of our expanding understanding of the breadth of this secosteroid action in calcium homeostasis. The role of CaBP in cellular calcium metabolism may be relevant in elucidating the physiological significance as well as the biochemical mode of action of vitamin D in the pancreatic secretion of insulin.

ANTHONY W. NORMAN
Department of Biochemistry, University of California, Riverside 92521

BARBARA J. FRANKEL
ANNELIESE M. HELDT
GERALD M. GRODSKY
Metabolic Research Unit, School of Medicine, University of California, San Francisco 94143

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Factors Influencing the Inhibitory Effect of Selenium on Mice Inoculated with Ehrlich Ascites Tumor Cells

Abstract. *Selenium, administered to mice with Ehrlich ascites tumors, effectively limited tumor growth. The response was dependent on the chemical form and dose of selenium administered. At the doses administered, there were no detectable adverse effects to the host.*

Selenium occurs naturally in many foods. As early as 1957, selenium was considered an essential nutrient (*1*), yet not until 1979 was a recommended dietary allowance for this metallic element proposed. Recent epidemiological evidence shows a negative correlation between selenium intake and tumor incidence in man (*2–8*). Also, selenium has been shown to be capable of inhibiting chemically induced, transplantable, and spontaneous tumor growth (*9–12*). Numerous factors may influence the ability of selenium to retard tumor incidence and growth. The present studies were designed to determine the influence of the chemical form and concentration of various selenium compounds on the growth of transplantable Ehrlich ascites tumor cells (EATC).

The tumor cell line was maintained in our laboratory by transplantation into Swiss/ICR mice at 14-day intervals. On day 0 of the experimental period, ascites tumors were removed from a stock animal and diluted in Krebs-Ringer-phosphate (KRP) solution consisting of 0.085*M* Na_2HPO_4, 0.016*M* NaCl, 0.005*M* KCl, and 0.0013*M* $MgSO_4 \cdot 7H_2O$ (*p*H 7.4). The cells were counted with a hemocytometer, and their viability was estimated by trypan blue dye exclusion (*13*). Cell viabilities were at least 98 percent. Each inoculum contained approximately 5×10^5 living EATC.

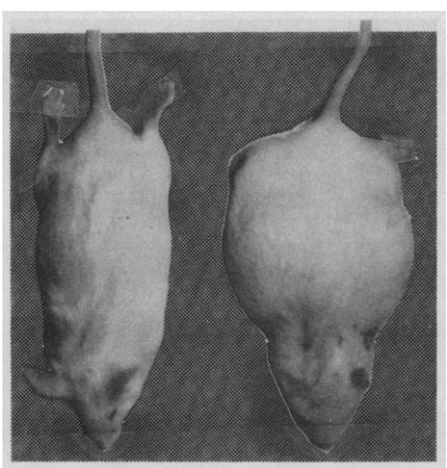

Fig. 1. Effects of treating or not treating EATC-inoculated mice with selenium. The mouse on the left received sodium selenite (1 μg per gram of body weight) and gained only 1.3 g in 21 days. The mouse on the right received KRP solution and gained 22.4 g.

Male Swiss/ICR mice weighing 20 to 22 g were used in the studies. They had free access to Purina mouse chow and distilled water and were housed in shoebox cages in groups of five. On day 0, the mice were inoculated with EATC and given intraperitoneal injections of KRP or a selenium compound dissolved in KRP. In general, each treatment was administered to ten EATC-inoculated mice and three noninoculated mice. The selenium (2.0 μg per gram of initial body weight) was administered as selenium dioxide, sodium selenite, sodium selenate, selenocystine, or selenomethionine. Additional injections followed on days 1, 3, 5, 7, 9, 12, 15, 18, and 21 (approximately 0.1 ml was administered per injection). The treatments were assigned at random.

Twenty-one days after being inoculated with EATC, half of the selenium-treated mice were killed by cervical dislocation and autopsied to determine the presence or absence of ascites tumors. The remaining EATC-treated mice were maintained without further selenium treatments for 21 more days and then were killed. On days 21 and 42, no tumors were visually evident in any of

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Table 1. Effect of various selenium compounds on mice with and without tumors. Each treatment was administered to ten EATC-inoculated mice and three noninoculated mice. All selenium compounds were administered at a dose of 2 μg per gram of initial body weight. Values with unlike superscripts differ at $P < .01$.

Treatment	Mean weight gain in mice without tumors (g)	Tumor-bearing mice		
		Mean weight gain (g)	Mean weight of small intestine (g)	Tumor incidence
KRP	10.7	17.3[a]	3.78[b]	10/10
Selenium dioxide	9.3	10.8[b]	2.46[c]	0/10
Selenium selenite	9.8	8.9[b]	2.58[c]	0/10
Selenium selenate	8.2	8.0[b]	2.49[c]	0/10
Selenomethionine	8.9	8.3[b]	2.50[c]	0/10
Selenocystine	8.9	9.1[b]	2.41[c]	0/10
Pooled S.E.M.*	± 1.06	± 1.20	± 0.28	

*Standard error of the mean.

the selenium-treated mice; however, all KRP-treated control animals had massive ascites tumors (Fig. 1 and Table 1). Analysis of variance showed that the weight gained by all selenium-treated mice was significantly less ($P < .01$) than that gained by KRP-treated controls, which is indicative of reduced ascites tumor development. Selenium in the forms administered did not significantly alter the growth of tumor-free mice, which was comparable to that of tumor-inoculated mice receiving the same selenium compound. Thus selenium may be valuable therapeutically without apparent ill consequences to the host.

Complete small intestines, livers, and spleens were removed from all mice at death and weighed. Selenium treatment had no significant effect on liver and spleen weights, but small intestine weights in all selenium-treated groups were significantly lower than those in tumor-inoculated, KRP-treated controls ($P < .01$). The amount of DNA, RNA, lipid, and protein per gram of small intestine was not significantly altered by selenium treatment. These data suggest that selenium may have an inhibitory effect on the growth of rapidly dividing cells.

In subsequent studies, we determined the effects of reducing the dose of selenium to 1 μg per gram of initial body weight. Again, selenium dioxide, sodium selenite, sodium selenate, and selenocystine completely inhibited formation of any visible tumor (Table 2). However, selenomethionine was not effective at this dosage. There were considerable weight gains, indicating progressive ascites tumor development in both KRP- and selenomethionine-treated mice.

Table 2. Effect of two different doses of various selenium compounds on mice with and without tumors. Most treatments were administered to five EATC-inoculated mice and three noninoculated mice. Values with unlike superscripts differ at $P < .01$.

Treatment	Dosage (micrograms per gram of body weight)	Mean weight gain in mice without tumors (g)	Tumor-bearing mice		
			Mean weight gain (g)	Tumor incidence	
				Ascitic	Solid
KRP		5.4	12.0[a]	5/5	0/5
Selenium dioxide	1.0	5.7	6.9[b]	0/5	0/5
Selenium selenite	1.0	5.0	5.8[b]	0/5	0/5
Selenium selenate	1.0	4.4	4.6[b]	0/5	0/5
Selenomethionine	1.0	6.2	12.3[a]	5/5	0/5
Selenocystine	1.0	6.1	6.1[b]	0/5	0/5
Pooled S.E.M.*		± 0.83	± 1.11		
Selenium dioxide	0.25	5.1	7.0[b]	0/4	1/4
Selenium selenite	0.25	5.6	7.7[b]	0/3	0/3
Selenium selenate	0.25	4.6	5.2[b]	0/5	2/5
Selenomethionine	0.25	5.2	12.4[a]	5/5	0/5
Selenocystine	0.25	4.6	7.8[b]	2/5	1/5
Pooled S.E.M.*		± 0.97	± 0.89		

*Standard error of the mean.

We also administered selenium at a dose of 0.25 μg per gram of initial body weight. All the selenomethionine-treated mice developed ascites tumors at this dosage (Table 2). Sodium selenite again completely inhibited formation of any visible tumor, as did selenium dioxide. However, at autopsy a solid tumor 2 to 3 mm in diameter was found subcutaneously at the site of the initial EATC inoculation in one of the mice treated with selenium dioxide and in two of the mice treated with sodium selenate. (Subcutaneous solid tumors developing in mice not treated with selenium attained a size of 2 to 3 cm in diameter during a 21-day study.) Two of the five animals treated with selenocystine had ascites tumors, and one had a subcutaneous solid tumor 1.0 to 1.5 cm in diameter. The volume of ascitic fluid in these two animals (0.5 to 1.0 ml) was considerably less than that in KRP-treated controls (4 to 6 ml).

Reports suggesting that selenium is a carcinogen have been severely criticized (*5*, *14*). Schroeder and Mitchener (*15*) found that administering sodium selenite or sodium selenate to rats throughout their lives had no detectable carcinogenic effect. Indeed, the evidence now suggests that selenium is anticarcinogenic. The data of Poirier and Milner (*11*) show that various forms of selenium can inhibit the growth of EATC in vitro. Complete inhibition of tumor development was observed for all selenium compounds administered at a dose of 2 μg per gram of body weight in the present studies. A wide range of doses of selenium dioxide, sodium selenite, and sodium selenate completely inhibited visible ascites tumor growth in mice in vivo.

Whanger *et al.* (*16*) showed that selenomethionine is actively transported, whereas selenite and selenocystine are not. Our data indicate that permeability may be a factor in the efficacy of the various selenium compounds in reducing tumor growth. Furthermore, selenium may alter tumor growth selectively, since it had no significant effect on the growth of the host animals. Future research may reveal selenium's mode of action and other factors influencing its efficacy as an antitumor agent.

GLENN A. GREEDER
J. A. MILNER
Department of Food Science, University of Illinois, Urbana 61801

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Dexamethasone Fails to Suppress β-Endorphin Plasma Concentrations in Humans and Rhesus Monkeys

Abstract. *In humans and rhesus monkeys, dexamethasone decreased concentrations of plasma cortisol but did not alter circulating β-endorphin immunoreactivity. Contrary to current theory suggesting that pituitary β-endorphin and adrenocorticotropic hormone are controlled by identical regulatory mechanisms for synthesis and release, our evidence suggests that in higher primates the established glucocorticoid feedback mechanism for the adrenocorticotropic hormone-cortisol system does not regulate β-endorphin secretion in the same way.*

Rats subjected to acute stress by limb fracture or footshock show concurrent increases in plasma adrenocorticotropic hormone (ACTH) and β-endorphin, whereas hypophysectomized rats fail to show this response (*1*). In addition, rats treated with dexamethasone show a concomitant decrease in plasma ACTH and β-endorphin (*1*). These observations are the foundation of a current hypothalamic-pituitary stress hypothesis suggesting that ACTH and β-endorphin are released simultaneously from the pituitary gland and that the regulatory mechanisms (hypothalamic releasing factors and glucocorticoid feedback) involved in the secretion and biosynthesis of both neuropeptides are common and identical.

Tissue culture experiments from normal mouse pituitary gland and a mouse pituitary tumor cell line (AtT-20) also support this hypothesis (*2*). A common polypeptide precursor of ACTH and β-lipotropin (pro-opiocortin) has been identified by using AtT-20 cells known to secrete ACTH, β-lipotropin, and β-endorphin, the last being a fragment of β-lipotropin (amino acid residues 61-91) (*2*). When these cultures are exposed to the synthetic glucocorticoids dexamethasone and prednisolone, the secretion of β-endorphin from these cells decreases in a dose-dependent fashion (*3*).

By using a dose of dexamethasone that markedly reduces plasma cortisol and ACTH in normal humans for up to 24 hours (*4*), we investigated the proposed common regulatory mechanism for the pituitary β-endorphin and ACTH-cortisol systems in humans and rhesus monkeys. One milligram of dexamethasone was given orally to nine adult humans (seven males and two females) who had no medical illnesses and were taking no medications. The single dose was given at 2300 hours as part of the standard overnight dexamethasone suppression test (*5*). After an overnight fast, blood samples were obtained from human subjects at 0800 hours on the mornings before and after dexamethasone was administered. In addition, nine adult male rhesus monkeys in individual cages were given an equivalent dose of dexamethasone, 0.017 mg per kilogram of body weight, intramuscularly, at 2300 hours. On the night before the baseline blood samples were drawn, the monkeys were given saline intramuscularly at 2300 hours as a control procedure (*6*).

The results shown in Fig. 1 indicate that in both humans and monkeys a reduction, averaging 75 percent, in plasma cortisol concentrations occurred 9 hours after dexamethasone administration. In contrast, plasma β-endorphin immunoreactivity remained essentially unaffected by dexamethasone administration in humans and monkeys.

Two differences between our experiment and the previously described rat stress experiment (*1*) in which dexamethasone suppressed both ACTH and β-endorphin may account for the discrepant results. First, the regulatory mechanisms for the synthesis and secretion of β-endorphin in higher primates may differ from those in rodents. Rodents, unlike humans, have a pituitary gland with a functional intermediate lobe thought to be important in the conversion of β-lipotropin to β-endorphin (*7*). Second, in the studies with rats, dexamethasone was administered over a 12-day period in doses more than 100 times greater than those sufficient to suppress stress-mediated increases in corticosterone concentrations in adult rats, and 5000 times greater than those required to suppress the rat corticosterone circadian cycle (*8*). In contrast, in our study, humans and monkeys were given a single low dose of dexamethasone sufficient to suppress ACTH and cortisol secretion.

Although a dissociation between β-endorphin and cortisol release has not been reported previously, dexamethasone given to seven normal human subjects at midnight suppressed plasma ACTH and β-lipotropin to undetectable levels but did not alter the amount of cir-

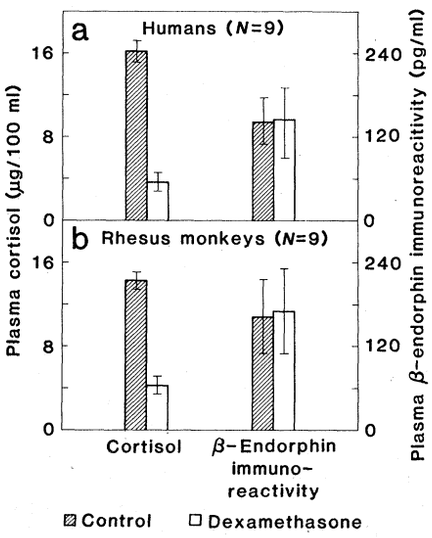

Fig. 1. The mean plasma cortisol concentrations ± standard deviation before and after dexamethasone administration were, respectively, 16.21 ± 4.88 and 3.68 ± 4.63 μg/100 ml in humans and 14.28 ± 4.26 and 4.34 ± 3.5 μg/100 ml in monkeys. In both humans and monkeys the cortisol concentrations after dexamethasone administration were significantly different from those before treatment ($P < .001$, two-tailed paired t-test). In the humans the baseline mean β-endorphin immunoreactivity value was 143.2 ± 34.1 pg/ml, and after dexamethasone treatment it was 145.6 ± 55.4 pg/ml; the immunoreactivity of the monkeys increased from a mean value of 163.3 ± 52.7 to 171.4 ± 61.5 pg/ml. Plasma β-endorphin immunoreactivity and cortisol determinations were performed with radioimmunoassay kits (New England Nuclear) with antiserum from rabbits prepared against synthetic human β-endorphin (*13*) and cortisol 21-succinyl bovine albumin, respectively (*14*). The antibody for β-endorphin demonstrates a 50 percent cross-reactivity with β-lipotropin, but less than 0.01 percent with α-endorphin and α-melanocyte stimulating hormone, and less than 0.004 percent with [Leu]enkephalin and [Met]-enkephalin.

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