$1,25(OH)_2D_3$ given to hens by itself cannot support normal embryonic development and hatchability in chicks. However, our results also show that 25-OH-D_3 by itself is fully capable of carrying out this function. Furthermore, the results with 24,24-F_2-25-OH-D_3 provide strong evidence that 24-hydroxylation of 25-OH-D_3 is not required as well. Thus, 25-OH-D_3 itself or some other unknown metabolite must be normally responsible for this function.

It is not clear why the embryos do not survive if their mothers do not have 25-OH-D_3. It may be that $1,25(OH)_2D_3$ is not transferred or stored in egg yolk. The requirement may be specifically for 25-OH-D_3 and may represent a phylogenetic indication of a function for 25-OH-D_3 itself. Which of these possibilities is correct will undoubtedly become apparent on continued investigation.

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Cysteamine: A Potent and Specific Depletor of Pituitary Prolactin

Abstract. *Cysteamine rapidly reduces the concentration of prolactin in pituitary tissue in vivo and in vitro. The effect is dose-dependent, reversible, and cannot be accounted for by prolactin release. Cysteamine does not appear to exert its effect through dopamine receptors and does not alter lactotrope morphology, as determined by electron microscopy.*

Cysteamine (2-aminoethanethiol) is a sulfhydryl drug with both clinical and experimental applications. Clinically, cysteamine has been used in the treatment of paracetamol (acetaminophen) poisoning (*1*) and nephropathic cystinosis (*2*). Experimentally, it has been shown to be radioprotective (*3*) and to be a duodenal ulcerogen in rats (*4*).

Szabo and Reichlin (*5*) found that cysteamine, administered orally to rats, rapidly and selectively depletes the gastrointestinal tract, hypothalamus, and plasma of immunoreactive somatostatin. We have since shown that cysteamine (300 mg/kg, subcutaneously) reduces the concentration of somatostatin throughout the central nervous system, especially in the hypothalamus (*6*). This effect is reversible, with the level of somatostatin returning to normal within 1 week.

Since somatostatin plays an important role in neuroendocrine regulation as a physiological inhibitor of the secretion of growth hormone (GH) and thyroid-stimulating hormone (TSH) (*7*), we investigated the effects of cysteamine administration on neuroendocrine function. We now report that the most striking effect of cysteamine on the neuroendocrine axis is on prolactin secretion.

Male Long-Evans hooded rats (Charles River) weighing 275 to 300 g were injected with cysteamine (300 mg/kg, subcutaneously) between 900 and 1000 hours and were killed at the intervals indicated in Table 1 (*8*). Within 2 hours of drug administration, prolactin concentrations in the anterior pituitary and serum (*9*) were reduced to less than 10 and 2 percent of control values, respectively. Prolactin levels in the serum and anterior pituitary were decreased for 8 hours, with serum levels returning to normal within 24 hours and pituitary levels within 72 hours. Cysteamine had no significant effect on the content of GH or TSH in the anterior pituitary.

Table 1. Time course of the effects of cysteamine (300 mg/kg, subcutaneously) on the concentration of prolactin in rat anterior pituitary and serum. Values are means ± standard errors for six animals per group. Control animals received 0.1*M* NaCl.

Group	Prolactin in anterior pituitary (μg/mg protein)	Prolactin in serum (ng/ml)
	2 hours	
Control	4.80 ± 0.77	55.9 ± 20.1
Cysteamine	0.57 ± 0.03*	1.1 ± 0.1*
	4 hours	
Control	8.67 ± 1.87	64.4 ± 16.5
Cysteamine	0.69 ± 0.08*	1.1 ± 0.1*
	8 hours	
Control	11.68 ± 2.25	78.8 ± 21.9
Cysteamine	0.82 ± 0.06*	1.2 ± 0.2*
	24 hours	
Control	7.23 ± 0.93	59.2 ± 21.6
Cysteamine	4.31 ± 0.50*	33.9 ± 8.7
	72 hours	
Control	10.15 ± 1.43	83.1 ± 14.3
Cysteamine	10.63 ± 2.30	49.5 ± 11.1
	1 week	
Control	5.45 ± 1.00	42.8 ± 11.7
Cysteamine	5.90 ± 0.50	43.3 ± 7.3

*Significantly different from corresponding control value at $P < .05$ (*9*).

Table 2 shows the response of prolactin to different doses of cysteamine. The drug produced a dose-dependent decrease in the concentration of prolactin in the anterior pituitary, with maximal effects observed at doses of 90 and 300 mg/kg. The median effective dose was approximately 30 mg/kg. The content of GH or TSH in the anterior pituitary was not affected by any dose of cysteamine.

These data indicate that cysteamine is a potent depletor of prolactin in vivo and that its effects occur rapidly and are reversible. In a previous study (*10*) we showed that luteinizing hormone but not follicle-stimulating hormone was slightly reduced in the anterior pituitary of animals given cysteamine subcutaneously at a dose of 300 mg/kg. However, lower doses of cysteamine (less than 200 mg/kg) did not affect the concentration of luteinizing hormone in the anterior pituitary. Thus, prolactin appears to be the anterior pituitary hormone most sensitive to the effects of cysteamine.

We next investigated the effect of cysteamine on dispersed anterior pituitary cells in culture (*11*). Pituitaries were harvested from male CD rats (Charles River) weighing 225 to 250 g; all tests were done on day 4 or 5 in culture. Cysteamine caused a dose-dependent decrease

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in prolactin in the medium and the cells, with a median effective concentration of 0.3 m*M* (Fig. 1a). No effect was observed on the GH content in the medium or the cells at any dose. The depletion of prolactin was maximal 1 hour after the administration of 0.7 m*M* cysteamine and was reversible, with cellular prolactin concentrations returning toward normal within 24 hours (*12*).

These data support the observation that cysteamine can decrease anterior pituitary prolactin in vivo and demonstrate that the depleting effect is not the result of prolactin release from lactotropes. The parallel reduction in the concentration of prolactin in both the cells and medium suggests that cysteamine may disrupt prolactin synthesis, storage, or degradation in the cell, perhaps through an intracellular digestive process.

There is evidence that lysosomes are important in the regulation of prolactin secretion (*13*). Dopamine increases lysosomal enzyme activity in dispersed anterior pituitary cells in culture, in part explaining the mechanism by which dopamine suppresses prolactin secretion (*14*). Dopamine is thought to be the hypothalamic prolactin-inhibiting factor acting through specific receptors on lactotropes (*15*). We therefore investigated whether the action of cysteamine is mediated by pituitary dopamine receptors or is accompanied by an alteration of lactotrope morphology, manifested by disruption of prolactin secretory granules or by increased lysosomal activity.

Dispersed anterior pituitary cells were incubated for 1 hour with 10^{-5} or 10^{-7}*M* spiperone or domperidone (*16*), two potent antagonists of dopamine receptors. Then 1.0 m*M* cysteamine was added and the cells were incubated for an additional hour. Neither spiperone nor domperidone prevented the cysteamine-induced decrease in anterior pituitary prolactin (Fig. 1b). These findings suggest that cysteamine acts on the lactotrope through a mechanism other than the dopamine receptor. Electron microscopic examination (*17*) of cysteamine-treated lactotropes at 1, 2, and 24 hours showed no morphological change. We observed normal-appearing prolactin secretory granules at each interval and saw no morphological evidence of lysosomal induction (that is, the number of multivesicular bodies in the lactotrope was not increased and there was no fusion of secretory granules to lysosomes). Thus cysteamine probably does not reduce the concentration of prolactin in cells by inducing intracellular digestive processes.

Table 2. Effects of various doses of cysteamine on the concentration of prolactin in rat anterior pituitary and serum. The animals were killed 4 hours after the subcutaneous administration of cysteamine. Values are means ± standard errors for ten rats per group. Control animals received 0.1*M* NaCl.

Cysteamine dose (mg/kg)	Prolactin in anterior pituitary (μg/mg protein)	Prolactin in serum (ng/ml)
Control	4.6 ± 0.7	8.2 ± 4.1
3.0	5.3 ± 0.9	3.1 ± 1.1
9.0	3.5 ± 0.4	4.7 ± 2.3
30.0	2.0 ± 0.2*	3.7 ± 1.3
90.0	0.57 ± 0.07*	0.44 ± 0.10*
300.0	0.26 ± 0.03*	0.30 ± 0.02*

*Significantly different from corresponding control value at $P < .05$. Differences in control values for serum prolactin between Tables 1 and 2 reflect the use of different rat prolactin reference preparations in the radioimmunoassay.

We next examined the possibility that cysteamine destroys immunologically active prolactin by forming mixed disulfides with cysteine residues in the prolactin molecule. There is precedent for such a reducing effect of cysteamine on disulfide bridges in patients with hereditary cystinosis (*2*). Since the immunological and biological activity of prolactin depends on the three disulfide bridges remaining intact (*18*), disruption of the bridges by cysteamine could explain the loss of prolactin immunoreactivity we observed. However, incubation of purified prolactin with cysteamine under culture conditions identical to those under which the anterior pituitary cells were incubated led to no loss of prolactin immunoreactivity. Furthermore, we have found that cysteamine, added to radioimmunoassay tubes at concentrations 30 times higher than those used in this study, does not interfere with prolactin immunoreactivity. However, this does not exclude the possibility that cysteamine can alter prolactin immunoreactivity in the lactotrope.

The observation that cysteamine disrupts pituitary prolactin stores by circumventing dopamine receptors and does not destroy lactotropes indicates that cysteamine may be useful in treating

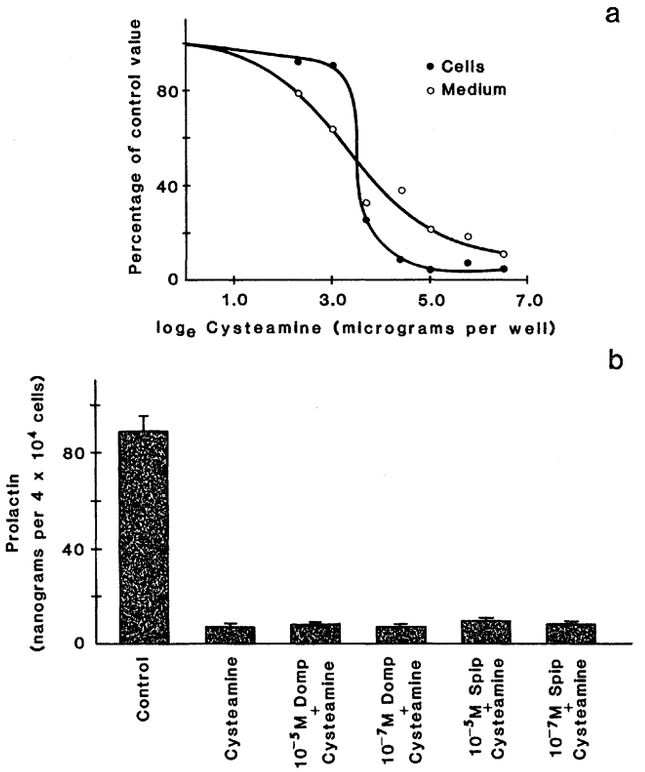

Fig. 1. (a) Effects of various doses of cysteamine on prolactin secretion in vitro. Each data point represents the mean of three determinations. Anterior pituitary cells were plated at a density of 4×10^4 cells per well in 1.0 ml of Dulbecco's modified Eagle's medium (DMEM) with glucose (4500 mg/liter), 10 percent fetal calf serum, 2.0 m*M* glutamine, 0.1 m*M* nonessential amino acids, penicillin, and streptomycin. The cells were incubated at 37°C in an incubator (National Appliance Company) under a water-saturated atmosphere of 5 percent CO_2 and 95 percent air. Just prior to experimentation, the cells were washed twice with Hepes buffer containing 0.1 percent bovine serum albumin (BSA). Test incubations were carried out in DMEM containing all of the above additives except fetal calf serum, for which 0.1 percent BSA was substituted. Neutralized cysteamine hydrochloride was added in a volume of 10 μl and the cells were incubated for 1 hour. At the end of the incubation period the medium was drawn off and immediately frozen. Cells were disrupted by sonication for 10 seconds in 2.0 ml of 0.1*M* borate buffer (*p*H 9.0) and then frozen. (b) Effects of dopamine receptor blockade on the cysteamine-induced decrease in pituitary prolactin. Values represent the mean of three determinations. Spiperone (*Spip*) and domperidone (*Domp*) were dissolved in 0.1*M* HCl. The drugs or their corresponding control vehicle were added in a volume of 10 μl. Neither of the two dopamine receptor antagonists affected prolactin levels when added alone. Since the control values were not significantly different from each other (one-way analysis of variance), they were combined for clarity.

patients with hyperprolactinemia. Up to one-quarter of such patients harbor a pituitary adenoma (*19*). Surgery, radiotherapy, or administration of dopamine receptor agonists have been the usual forms of treatment for hyperprolactinemia. Bromocriptine is the drug most widely used; it restores normal gonadal function and fertility in nearly 80 percent of the hyperprolactinemic patients on which it is used. There is evidence that bromocriptine can also reduce pituitary tumor size (*19*).

Despite the success of bromocriptine, its use is limited; some patients fail to respond to it or develop undesirable side effects, such as gastrointestinal upset and postural hypotension. Cysteamine or similar drugs may prove beneficial in treating such patients by reducing prolactin secretion.

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8. Cysteamine (Sigma) doses are expressed as milligrams of the HCl salt, which was dissolved in distilled water and neutralized with 1.0*N* NaOH. The dose (300 mg/kg) was selected for the time course study because we have found (*6*) that this dose, administered subcutaneously, produces the greatest depletion of immunoreactive somatostatin in the brain without evidence of side effects, such as the development of duodenal ulcers or the induction of seizures. The animals were decapitated and the trunk blood and anterior pituitaries were harvested. Plasma was separated from the blood cells by centrifugation and stored at −30°C until being assayed for prolactin. The anterior pituitaries were sonicated for 30 seconds in 1.0 ml of phosphate-buffered saline and a portion of the supernatant was removed for the determination of prolactin. Protein levels were determined on the supernatant by the method of O. H. Lowry, J. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)].
9. Serum and anterior pituitary samples were assayed in duplicate for prolactin with materials supplied by A. F. Parlow and the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD). All prolactin values are expressed in terms of the NIAMDD reference preparation Rat-PRL-RP-1 (Table 1) and Rat-PRL-RP-2 (Table 2 and in vitro data). All samples from a single experiment were assayed in the same assay to avoid interassay variations. The range of detectability in 50 μl of undiluted plasma was 1.0 to 250 ng/ml for Rat PRL-RP-1 and 0.25 to 50 ng/ml for Rat-PRL-RP-2. The significance of differences was tested with the Mann-Whitney *U* Wilcoxon nonparametric test. Significance was set at $P < .05$.
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16. The ability of spiperone and domperidone to bind dopamine receptors was determined by identifying the dose of the receptor antagonist that could most effectively block the inhibitory effects of two dopamine receptor agonists, apomorphine and bromocriptine, on prolactin release in vitro at their (the agonists') maximal effective concentrations, and then increasing the dose of the antagonists by two and four orders of magnitude.
17. The rats were given cysteamine (300 mg/kg, subcutaneously) and anesthetized with pentobarbital (50 mg/kg, intraperitoneally) 1, 2, or 24 hours later. Then they were perfused for 15 to 20 minutes with warmed 2.0 percent paraformaldehyde and 2.5 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer (*p*H 7.4). The pituitaries were later removed, bisected, postfixed in 1.0 percent osmium tetroxide, and embedded in Epon.
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Cure of Mice Infected with *Trypanosoma rhodesiense* by *cis*-Diamminedichloroplatinum (II) and Disulfiram Rescue

Abstract. *Mice infected with* Trypanosoma rhodesiense *were treated concurrently with* cis-*diamminedichloroplatinum (II) (DDP), disulfiram, and hydration. Most of the mice (92.5 percent) were cured; inoculation of blood or suspensions of brain or heart from these animals did not produce disease in recipient mice. The dose of DDP needed to eliminate the trypanosomes, 3 milligrams per kilogram of body weight per day for 7 days, was lethally toxic unless the animals received disulfiram orally and subcutaneous injections of physiologic saline, which reduced the acute renal necrosis caused by DDP alone. Some mild to moderate reversible renal damage was noted upon pathologic examination of the treated mice.*

Several antineoplastic compounds show antitrypanosomal activity in vivo (*1, 2*). One of these agents is *cis*-diamminedichloroplatinum (II) (DDP). Experiments with mice indicated that, although infected animals were not completely cured by DDP, the drug did prolong their life (*1*). This suggested that the DDP had trypanocidal activity but that the doses administered were inadequate to eradicate all of the infecting parasites.

Methods have recently been devised to ameliorate or ablate the adverse effects of DDP, primarily renal tubule toxicity, previously observed at therapeutic doses (*3*). These methods include hydration and administration of compounds thought to preferentially block DDP's toxic effects without blunting its antineoplastic action (*4*). Diethyldithiocarbamate, a metabolite of disulfiram (Antabuse), is perhaps the best characterized of these substances. Its protective action is thought to derive from the chelation of DDP prior to the generation of irreversible nephrotoxicity. These protective agents allow greater doses of DDP to be administered without concomitant increases in nephrotoxicity, thus enhancing DDP's therapeutic index. They also make it possible to examine the curative potential of DDP in trypanosome-infected animals treated with substantially increased doses.

In studies of the effects of DDP on mice (ICR/Ha Swiss) infected with *Trypanosoma rhodesiense*, we first measured the activity of the drug against the infecting organism in vitro. Parasites were collected from male Walter Reed rats by cardiac puncture and isolated by using a dimethylaminoethyl cellulose anion exchange column. Elution was effected with 0.1*M* phosphate-buffered sa-

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