leased by H_2SO_4, trapped with hyamine hydroxide and estimated using a scintillation counter (Intertechnique).

Compound 2, which obviously cannot be a substrate, was the only compound showing inhibitory properties; K_i values obtained through Lineweaver-Burk plots (fig.) were one order of magnitude greater than the K_m of both *E.coli* GAD (K_m = 0.9 mM; K_i = 10 mM) and rat brain GAD (K_m = 1.3 mM; K_i = 10.5 mM). Our results indicate that binding of the α phosphonic group at the active site of both enzymes is weakened by steric hindrance.

Compound 3, despite the presence of an α-aminocarboxylic group, was neither a substrate nor an inhibitor. Negative results were also obtained with 4 and with two phosphonic analogues of aspartic acid, 3-amino-3-phosphonopropionic and 2-amino-3-phosphonopropionic acids: the brain enzyme which recognizes L-aspartic acid as a substrate was not inhibited by the two last compounds tested at a concentration 30-fold higher than that of the substrate. Moreover, the inertness of compound 3 may emphasize the importance of the γ-carboxylic group of glutamic acid for enzyme recognition. We have investigated the role of this group by studying the effect on decarboxylase activity of 2-amino-4-(methylphosphino)-butyric acid, also known as phosphinothricin[9], homocysteic acid and norvalin, compounds differing from glutamic acid by the replacement of its γ-COOH group by $-PO(OH)(CH_3)$, $-SO_3H$ and $-CH_3$ respectively. The strict specificity of *E.coli* GAD towards the γ-carboxyl group was confirmed by the lack of inhibitory effect of the above-mentioned substances; this may be related to the recent observation of Vospel et al.[10] that this acidic group is involved in a linkage with an arginine residue in the catalytically active form of *E.coli* GAD. Unlike the bacterial enzyme, brain GAD was able to recognize other acidic groups in the same position: 2-amino-4-(methylphosphino) butyric acid was a potent competitive inhibitor with a K_i of 2.2 mM, a value very close to the K_m for glutamate; L-cysteic and L-homocysteic acids, used in a molar ratio |inhibitor|/|substrate|=20, led to inhibition values of 60 and 40% respectively. L-Norvaline was inert towards the two enzymes. It may be emphasized that all the inhibitory molecules tested possess an ω-mono-anionic group like the substrate, a fact which may explain the inertness of 3 and 4 towards brain GAD. However, work in an other field has shown that compound 3 is able to interfere with the neuronal glutamate receptor[11].

The dialanyl derivative of phosphinothricine is a natural compound which exerts its antibiotic properties against several microorganisms through the action of phosphinothricin on glutamine synthetase[9]; phosphinothricin is also a potent inhibitor of rat liver glutamine synthetase[12]. We have shown here another target site of this compound in eucaryotic enzymatic system.

1 We acknowledge the gift of a sample of phosphinothricin by Prof. Przemyslaw Mastalerz as well as the technical assistance of Denois.
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Number of dopamine neurons predicts prolactin levels in two inbred mouse strains

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Summary. Mice of the BALB/cJ strain have more dopamine neurons than mice of the CBA/J strain. We now report that BALB/cJ mice have less circulating and pituitary prolactin than CBA/J mice, a relationship expected from the difference in tuberoinfundibular dopamine neuron number.
Key words. Prolactin; inbred mouse strains; dopamine neurons; hypothalamus; pituitary.

Mice of the BALB/cJ strain display greater activity of the catecholamine synthesizing enzyme tyrosine hydroxylase (TH) in brain compared to another inbred strain of mice, CBA/J[1,2]. Histological analysis using antibodies to tyrosine hydroxylase to localize catecholamine cells has revealed that the difference in TH activity between these two strains is due entirely to a difference in number of dopaminergic cells. Mice of the BALB/cJ strain have 20–50% more TH staining neurons in each population of dopamine cells in the brain (i.e., A9, A10, A11, A12, A13, A14) compared to CBA/J mice[3,4]. The activity of TH per dopamine cell, as calculated from the activity of TH measured in vitro and the number of TH staining cells, is not different between the two strains[3]. The possibility that the release of dopamine may be greater in brains of BALB/cJ mice compared to CBA/J mice is supported by biochemical studies showing a greater dopamine synthesis and turnover rate in the BALB/cJ strain[5].

The difference in dopamine cell number raises the question of how these strains of mice may differ in physiological processes and behaviors, especially those involving dopamine neurons. Fink and Reis[6,7] compared these two mouse strains with respect to locomotor activity and behavioral responses to drugs which act via dopamine neurons. Their studies support the hypothesis that the level of dopaminergic neurotransmission in BALB/cJ mice is greater than in CBA/J mice. We have extended those findings by examining another process that is dependent upon dopamine neurons in the central nervous system, the regulation of prolactin release.

Methods. Male and female mice of the BALB/cJ and CBA/J strain were obtained from the Jackson Laboratory (Bar Har-

bor, ME) at six weeks of age. They were housed in groups of five under controlled conditions (12–12 h light-dark cycle, 22 °C) with ad libitum food and water for two weeks before they were used in experiments. At time of use in experiments, mice of each strain weighed 25–30 g. The first group of male mice and the female mice were sacrificed between 09.00 and 10.30 h; the second group of male mice were sacrificed between 14.30 and 15.30 h. Mice were rapidly decapitated and blood collected from the cervical wound into chilled test tubes. In addition, from the first group of male mice the entire pituitary gland was removed and the hypothalamus dissected from the rest of brain[4]; tissues were rapidly frozen on dry ice. Blood samples were centrifuged (3000 × g for 10 min) and the sera removed and frozen (−20 °C) until assayed. Pituitaries and hypothalami were stored at −70 °C.

Serum and pituitary prolactin levels were measured by radioimmunoassay (RIA) using reagents supplied by the National Institute of Arthritis, Metabolic, and Digestive Diseases. Pituitaries were homogenized in saline buffered with 10 mM sodium phosphate at pH 8.0. Aliquots of sera or pituitary homogenate were diluted to 400 µl with RIA buffer (0.01 M $NaPO_4$, 0.9% NaCl, 0.025 M EDTA, 0.1% BSA, 0.1% sodium azide, pH 7.5). The tubes were then incubated with 100 µl of a 1:50,000 dilution of rabbit antimouse-prolactin serum (AFP-131078) and 100 µl of I^{125} mouse prolactin (10,000 cpm). The I^{125} prolactin was prepared by the chloramine T method of Greenwood et al.[8] as described by Sinha et al.[9], using purified mouse prolactin supplied by NIAMDD (AFP-4111-E). Following an overnight incubation at room temperature, normal rabbit serum and goat antirabbit serum were added and the samples were incubated at 4 °C for an additional 24 h. The samples were centrifuged (3000 × g, 20 min), the supernatants aspirated, and the pellets counted for radioactivity. NIAMDD purified mouse prolactin (AFP-4111-E) was used as the standard; results are expressed as ng prolactin relative to this standard. The sensitivity of this assay is approximately 0.20 ng/tube. The intraassay coefficient of variation is less than 10%, and all samples from a single experiment were run in the same assay. Dilutions of pituitary homogenates or sera from both strains produced inhibition of antibody binding of I^{125} prolactin parallel to the mouse prolactin standard. This assay does not recognize mouse growth hormone.

Tyrosine hydroxylase activity was measured by the method of Coyle[10] as modified by Joh et al.[11]. Protein was measured in aliquots of homogenate from pituitary and hypothalamus by the method of Lowry et al.[12] using BSA as the standard.

Data are expressed as means ± SEM and the parameters for the two strains were analyzed by t-test (one-tailed, unpaired).

Results. The levels of prolactin in the blood and pituitary gland, as well as tyrosine hydroxylase activity in the hypothalamus, from BALB/cJ and CBA/J mice are presented in table 1. Prolactin levels are greater by approximately 20–30% in the CBA/J mice, in both blood and pituitary. The difference in pituitary prolactin levels is significant whether the prolactin content is expressed per gland or per mg protein, an estimate of pituitary weight. The slight difference in magnitude between the groups when expressed per gland compared to per mg protein reflects a slight difference in pituitary size between these two strains. The difference in both serum and pituitary prolactin level is very similar in magnitude, and reciprocally related to, the difference in tyrosine hydroxylase activity between the two strains.

Table 1. Serum and pituitary prolactin levels in BALB/cJ and CBA/J mice

	BALB/cJ	CBA/J	CBA/BALB
Serum prolactin (ng/ml)	7.8 ± 0.6	9.8 ± 0.6	1.26
Pituitary prolactin (µg/gland)	1.62 ± 0.05	2.03 ± 0.06	1.25
Pituitary prolactin (µg/mg protein)	5.84 ± 0.11	7.01 ± 0.12	1.20
Tyrosine hydroxylase activity (nmoles DOPA/h/mg protein)	2.56 ± 0.05	1.59 ± 0.03	0.62

Mice of each strain (n=10) were sacrificed between 09.00 and 10.30 h and blood, pituitary gland, and hypothalamus were collected. Prolactin was measured in pituitary gland and serum and tyrosine hydroxylase activity was assayed in hypothalamus. There was a significant difference ($p < 0.05$) between strains in each of these measures.

To confirm this difference in prolactin levels between these two strains, we repeated the experiment using male mice sacrificed at another time of day (14.30–15.30 h) and using female mice of the two strains. The female mice were sacrificed between 09.00 and 10.00 h since at this time, the stage of estrous cycle has little effect on plasma prolatin levels[13]. As shown in table 2, the strain difference in serum prolatin levels occurred in these two additional groups.

Discussion. These results demonstrate that serum and pituitary prolactin levels are inversely related to dopamine neuron number in two inbred strains of mice, BALB/cJ and CBA/J. As dopamine is known to inhibit the release and synthesis of prolactin[14–16], this is the expected relationship between prolactin levels and dopaminergic activity. This observation of more prolactin in mice of the CBA/J strain compared to mice of the BALB/cJ strain is consistent with the BALB/cJ strain have more hypothalamic dopamine neurons and releasing more dopamine.

That BALB/cJ mice have more brain dopamine neurons than CBA/J mice has been carefully documented[3,4]. The difference in dopamine neuron number is apparent in all dopamine cell groups (including the A12 group, which is involved in the regulation of prolactin release[14,15]), with BALB/cJ mice having 20–50% more dopamine neurons than the CBA/J mice. Fink and Reis[6,7] began examining the physiologic and behavioral consequences of this difference in dopamine neuron number by comparing these two mouse strains with respect to certain behaviors and responses to drugs which are thought to be mediated by dopaminergic neurotransmission. They reported that exploratory activity and several behavioral responses to amphetamine injection which are thought to be mediated by A9 and A10 dopamine neurons are greater in BALB/cJ mice than CBA/J mice. Our finding of a decreased pituitary and plasma level of prolactin in BALB/cJ mice compared to CBA/J mice is consistent with these results. As the role of dopamine function in the regulation of prolactin release is probably the most thoroughly studied and understood physiological action of dopamine, these data strengthen the hypothesis that dopaminergic neurotransmission is enhanced in the BALB/cJ mice compared to CBA/J mice.

These data can also be viewed as support for the hypothesis that serum prolactin levels are a valuable index of brain dopamine function. With only a 20–50% difference in dopamine neuron number, a detectable difference in serum prolactin levels was present. This could be taken as evidence in favor of the possibility that serum prolactin levels provide a sensitive index for diseases of central dopamine neurons.

It has been known for some time that serum prolactin levels vary considerably between inbred mouse strains[9,13]. Our results

Table 2. Serum prolactin levels in male and female mice of the BALB/cJ and CBA/J strains

	BALB/cJ	CBA/J	CBA/BALB
Males	6.4 ± 0.6	9.2 ± 1.3	1.43
Females	17.2 ± 3.0	25.1 ± 3.7	1.46

Female mice of each strain (n=10) were sacrified between 9.00 and 10.00 h. Male mice of each strain (n=8) were sacrificed between 14.30 and 15.30 h. Blood was collected and sera assayed for prolactin. There was a significant difference ($p < 0.05$) between the sexes of each strain.

suggest that differences in brain dopamine neuron number may underlie some of these strain differences in prolactin levels. Furthermore, since the incidence of mammary cancer among strains of mice is correlated to serum prolactin levels[17], it may be that brain dopamine neuron number can be related to the development of mammary cancer in some strains. In fact, BALB/cJ mice have a much lower incidence of mammary tumors than CBA/J mice[18].

In summary then, we have demonstrated that two strains of mice that differ in the number of dopamine neurons in the brain also differ in blood and pituitary prolactin levels in a manner predicted by their dopamine neuron number.

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Effect of tingenone, a quinonoid triterpene, on growth and macromolecule biosynthesis in *Trypanosoma cruzi*[1]

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Summary. Tingenone and horminone, two natural quinonoid substances, inhibited the in vitro growth of *Trypanosoma cruzi,* 30 μM drug concentration producing total inhibition of growth. Tingenone inhibited total uptake and incorporation of [^{3}H]thymidine, [^{3}H]uridine, L-[^{3}H]leucine into parasite macromolecules. Other quinonoids assayed were either less effective (abruquinone A) or even quite inactive (visminone B and ferruginin B). Investigation of several mechanisms for the cytotoxic action of tingenone pointed to the interaction with DNA as the most likely factor involved. Tingenone also inhibited the growth of *Crithidia fasciculata,* but the drug was significantly less active on this organism than on *T. cruzi.*
Key words. Trypanosoma cruzi; Crithidia fasciculata; tingenone; horminone.

Tingenone (maitenine; fig. 1) is a triterpene quinone-methide, isolated from various plants of the Celastracea and Hyppocrateacea families as a red-orange pigment, displaying antineoplastic activity[3-5]. The size and shape of the tingenone molecule are favorable for its inclusion within the narrow groove of DNA, and hydrogen bonds can be formed between the hydroxyl group of tingenone and the phosphate group of DNA[6]. In the present study we have investigated the action of tingenone and other natural quinonoid substances as inhibitors of growth and macromolecule biosynthesis in *Trypanosoma cruzi,* the agent of Chagas' disease. The same substances were tested on *Crithidia fasciculata,* a nonpathogenic flagellate which has been suggested for in vitro testing of potential trypanocides[7]. Studies on the mode of action of drugs on *T. cruzi* are of permanent interest because the identification of potential targets for chemotherapeutic attack may pave the way for the development of new agents for the treatment of Chagas' disease.

Materials and methods. Organism. T. cruzi (Tulahuen strain) was cultured for 3–4 days, at 28 °C, in a liquid medium composed of brain-heart infusion (Difco), 37 g; hemin, 20 mg, fetal bovine serum, 40 ml and water, to 1 l[8-10]. The cells were collected during exponential growth by centrifugation and resuspended in fresh warm medium at a concentration of 1×10^6 cells/min. Krebs-Ringer was used for resuspending the cells when uptake or incorporation of L-[^{3}H]leucine was to be determined. *Crithidia fasciculata,* anopheles strain ATCC 11745, was cultured as above. After 48 h culture, the cells were collected by centrifugation and washed with 0.154 M NaCl before the experiments.

Heart mitochondria electron transport particles were prepared as described by Turrens and Boveris[11].

Reagents. These were obtained from the following sources: [Methyl-^{3}H]thymidine (5 Ci/mmole), [5,6-^{3}H]uridine (40 Ci/mmol) and L-[4,4-^{3}H]leucine (40 Ci/mmole) (henceforth [^{3}H]thymidine, [^{3}H]uridine and L-[^{3}H]leucine, respectively) from the Radiochemical Centre, Amersham, UK; ascorbic acid, NADH, sucrose, mannitol, EDTA and antimycin, from Sigma Chemical Co, St. Louis, Mo, USA; tingenone[3], abruquinone A[12], ferruginin B[13], vismione B[14] and horminone[15] were

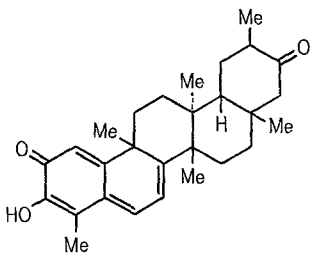

Figure 1. Tingenone: D:A-Friedo-24,30-dinoroleana-1(10),3,5,7-tetraene-2,21-dione, 3-hydroxy,-(20β)-.