was blocked by atropine but not by methylatropine thus excluding involvement of extracerebral mechanisms on the striatal DA release. The view that DA neurons receive an excitatory cholinergic influence is further supported by the findings that anti-acetylcholine agents decrease the DA turnover and counteract the enhanced liberation of the amine caused by neuroleptic drugs[14,15]. Conversely, cholinergic neurons in the striatum receive an inhibitory dopaminergic input: in fact, impairment of DA transmission by neuroleptic drugs results in an increased output of acetylcholine into the perfusate of the cat caudate nucleus and this effect is prevented or reversed by dopaminergic agents such as L-dopa or apomorphine[16].

The results obtained with oxotremorine together with the findings on acetylcholine release indicate a functional interconnection between cholinergic and dopaminergic neurons in the extrapyramidal system, which might mediate rapid feed-back mechanisms modulating the activity of these neurons[16]. For instance, the enhanced DA turnover caused by neuroleptic drugs may result not only from blockade of presynaptic DA receptors[17] but also from disinhibition of a cholinergic system which activates the dopaminergic neurons.

In conclusion, the present results show that the effect of drugs on the release of pg quantities of endogenous DA and NA from discrete brain areas can be directly investigated using the push-pull cannula perfusion technique. The output of catecholamines from various brain regions can also be measured in the unrestrained unanaesthetized cat by means of chronically implanted push-pull cannulae (in preparation), as previously described for acetylcholine release[18].

Zusammenfassung. Hirnregionen der gallamin-immobilisierten Katze wurde mittels «push-pull»-Kanülen perfundiert. Die im Perfusat freigesetzten endogenen Katecholamine wurden radioenzymatisch gemessen. Chlorpromazin, D-Amphetamin oder Oxotremorin (i.v.) erhöhten den «output» von Dopamin aus dem Nucleus caudatus und Chlorpromazin zusätzlich denjenigen von hypothalamischem Noradrenalin durch verschiedene Mechanismen.

K. G. LLOYD[19] and G. BARTHOLINI

Department of Experimental Medicine, F. Hoffmann-La Roche & Co. Ltd., CH-4002 Basel (Switzerland), 12 February 1975.

[14] G. BARTHOLINI and A. PLETSCHER, in *Advances in Biochemical Psychopharmacology* (Raven Press, New York 1972), vol. *6*, p. 65.
[15] N.-E. ANDÉN and P. BÉDARD, J. Pharm. Pharmac. *23*, 460 (1971).
[16] G. BARTHOLINI, H. STADLER and K. G. LLOYD, in *Frontiers in Catecholamine Research* (Eds. E. USDIN and S. H. SNYDER; Pergamon Press, Oxford 1973), p. 741.
[17] A. CARLSSON, in *Advances in Neurology* (Eds. P. F. MCDOWELL and A. BARBEAU; Raven Press, New York 1974), vol. 5.
[18] M. GADEA-CIRIA, H. STADLER, K. G. LLOYD and G. BARTHOLINI, Nature, Lond. *243*, 518 (1973).
[19] Present address: Department of Psychopharmacology, Clarke Institute of Psychiatry, 250 College St., Toronto, Canada.

Increase in Food Consumption and Growth after Treatment with Aminoguanidine

In an earlier study, we observed an increase in appetite in a patient with medullary carcinoma of the thyroid upon treatment with the diamine oxidase (DAO, histaminase) inhibitor, aminoguanidine[1]. DAO is produced in large amounts by this tumor[1-4], and aminoguanidine was administered to see if inhibition of DAO influenced tumor growth. During treatment, the patient noted an increase in her appetite, she ate well, her weight increased, although no regression of tumor was noted[1]. On the basis of this observation, further studies were undertaken in rats to see if aminoguanidine had a specific effect on appetite. In addition, the distribution of this drug and its effect on diamine oxidase activity in various tissues was studied.

Methods and materials. The patients included 2 subjects with widely disseminated medullary carcinoma of the thyroid who were chronically ill. The first was a 19-year-old white female. She received the cytotoxic agents, Cytoxan and Vincristine. She was subsequently treated with aminoguanidine, 30 mg/day (3 × 10 mg), for a period of 5 months and then for 2 months. Therapy was discontinued 2 weeks before her death. The second patient, a 45-year-old male, was admitted to NIH in an almost moribund condition. He received aminoguanidine, 30 mg daily, but died 17 days after admission to NIH while still on the drug. The history of these patients has been described in detail elsewhere as Patient 1 and 8[2].

For the animal studies, male Sprague-Dawley rats (Zivic Miller, Inc., Allison Park, Pa., average weight, 80 g) were housed, individually, in metal cages and were given water and powdered Purina Chow ad libitum. Food was supplied in stainless steel dishes with concave lids having an aperture of 3.4 cm diameter to prevent spillage. Room lighting (12 h on, 12 h off) and temperature (23–25 °C) were controlled. Aminoguanidine sulfate was administered orally by stomach tube in doses of 10 or 50 mg/kg daily in distilled water. Control animals received water alone. Measurement of food consumption and body weight were made daily.

At the end of each experiment, the animals were killed by decapitation. Tissues were rinsed briefly in water, blotted and frozen on dry ice for storage at −20 °C. Aminoguanidine was assayed by reaction with *p*-nitrobenzaldehyde as described by BEAVEN et al.[5]. Diamine oxidase activity was measured by the procedure of BEAVEN and JACOBSEN[6]. This procedure measures the release of tritiated water from side chain labeled β-^{3}H-histamine upon deamination.

Aminoguanidine sulfate and *p*-nitrobenzaldehyde were purchased from Eastman Kodak Company, Rochester, N.Y.; β-^{3}H-histamine was prepared from β-^{3}H-L-histidine as described previously[6].

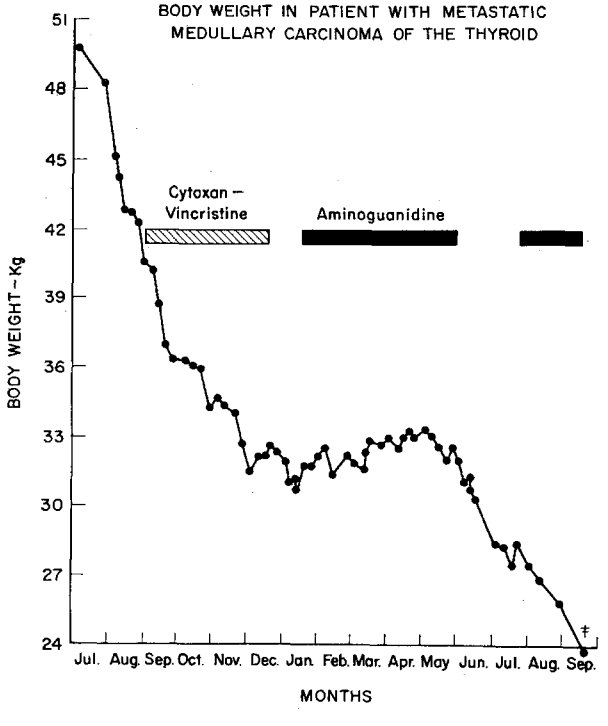
[1] S. B. BAYLIN, M. A. BEAVEN, K. ENGLEMAN and A. SJOERDSMA, New Engl. J. Med. *283*, 1239 (1970).
[2] S. B. BAYLIN, M. A. BEAVEN, L. M. BUJA and H. R. KEISER, Am. J. Med. *53*, 723 (1972).
[3] S. B. BAYLIN, M. A. BEAVEN, S. B. BAULIN, M. A. BLAUEN, H. R. KEISER, A. H. TASHJIAN Jr. and K. E. W. MELVIN, Lancet *1*, 455 (1972).
[4] H. R. KEISER, M. A. BEAVEN, J. DOPPMAN, S. WELLS, JR. and L. M. BUJA, Ann. intern. Med. *78*, 561 (1973).
[5] M. A. BEAVEN, J. W. GORDON, S. JACOBSEN and W. B. SEVERS, J. Pharmac. exp. Ther. *165*, 14 (1969).
[6] M. A. BEAVEN and S. JACOBSEN, J. Pharmac. exp. Ther. *176*, 52 (1971).

Table I. Effect of aminoguanidine on food consumption and weight gain of rats

Group	n	Total food consumption (g)		Total increase in body weight (g)		Increase in body weight as % of food consumed	
		Before Treatment	On	Before Treatment	On	Before Treatment	On
Experiment I							
Control (vehicle)	6	79 ± 7	471 ± 13	61 ± 10	135 ± 7	—	29
10 mg/kg	5	78 ± 5	507 ± 17	69 ± 8	148 ± 10	—	29
50 mg/kg	6	81 ± 3	514 ± 12[a]	66 ± 6	143 ± 6	—	28
Experiment II							
Control (vehicle)	11	79 ± 4	805 ± 21	49 ± 4	233 ± 9	49	29
10 mg/kg	11	79 ± 6	840 ± 19	46 ± 4	255 ± 10	58	30
50 mg/kg	10	76 ± 4	886 ± 29[a]	49 ± 3	261 ± 11[a]	65	29

Values are mean ± SEM. [a] Significant difference (p 0.05) from control values. Period before and period of treatment was 8 and 18 days, respectively for Experiment I and 5 and 34 days for Experiment II. The data show the food consumed and increase in weight before and during treatment with vehicle or aminoguanidine.

Results. During treatment with Cytoxan and Vincristine, the weight of Patient 1 declined from 50 to 30 kg (Figure). Within a week of treatment on aminoguanidine, the patient reported an unexpected improvement in appetite. She noted a particular craving for cakes and cinnamon buns. During this period her weight increased by 3.3 kg (Figure). Radiological examination indicated that tumor had spread into lung and bone[2], and aminoguandine therapy was discontinued. She again lost weight and, despite a brief period of treatment, she continued to lose weight until her death in the autumn of 1970 (Figure). The second patient was admitted while in the terminal phase of his disease. He showed no response to treatment with aminoguanidine, and the died within 2 weeks. The effect of aminoguanidine on the histaminase activity in tissues of these patients has been described[2]. No toxic or side effects of the drug were observed. In addition to these, we received a report (HILL, personal communication) of a patient with medullary carcinoma whose appetite increased while receiving aminoguanidine[7]. The physician was not aware of our observations at that time. We have not conducted further experiments in human subjects.



Reversal of decline in body weight in Patient 1 during aminoguanidine therapy. The patient received aminoguanidine, 30 mg t.i.d. orally, as described in text during the periods indicated. The patient died in mid-September with widely disseminated tumor.

In two studies in rats, food consumption and growth were increased after treatment with 10 and 50 mg/kg of aminoguanidine (Table I). These increases were statistically significant only with the 50 mg/kg dose of drug. Increased food consumption (1 to 2 g/day) was apparent after about 8 days and persisted throughout the course of treatment. At autopsy, the organs showed no gross pathological abnormalities. There were increases in the weights of heart, liver and perineal fat, but not when calculated in terms of percent of body weight (Table II). Aminoguanidine levels were high in kidney, intestine, salivary gland, liver and thymus and moderately high in serum. None was detectable in brain. Histaminase activity was reduced in all tissues after aminoguanidine. The reduction was most marked in tissues which normally had high activity (Table II).

Discussion. Relatively few compounds are available that stimulate appetite and increase weight. Androgenic steroids, such as methyltestosterone[8–10], have been used. These have obvious drawbacks because of undesirable side effects in women and children and repression of endogenous testosterone production in adult males. The antiserotonin compound, cyproheptadiene, produces

[7] C. S. HILL, JR., M. L. IBANEZ and N. A. SAMAAN, Medicine *52*, 141 (1973).

[8] K. KNOWLTON, A. T. KENYON, I. SANDIFORD, G. LOTWIN and R. FICKER, J. clin. Endocr. Metab. *2*, 671 *18*, 1043 (1942).

[9] J. S. BRADSHAW, W. E. ABBOTT and S. LEVEY, Am. J. Surg. *99*, 600 (1960).

[10] A. E. FRUEHAN and T. H. FRAWLEY, J. Am. med. Ass. *184*, 527 (1963).

Table II. Effect of aminoguanidine treatment on tissue weight, aminoguanidine and histaminase levels

Tissue	Tissue weight		
	Control	Aminoguanidine (g)	Increase (%)
Heart	1.13 ± 0.05 (0.31)[a]	1.22 ± 0.1 (0.29)	+ 8
Liver	12.8 ± 0.6 (3.31)	13.7 ± 0.4 (3.3)	+ 7
Fat (perineal)	3.22 ± 0.2 (0.83)	3.35 ± 0.2 (0.82)	+ 4
Whole Body	233 ± 9	261 ± 11	+12.1[b]

Tissue	Histaminase activity (units/g)			Aminoguanidine levels (μg/g)	
	Control	Aminoguanidine	Decrease (%)	Control	Aminoguanidine
Thymus	274 ± 7	12 ± 3	−96[b]	—	16.5 ± 2.5
Intestine	672 ± 132	45 ± 16	−93[b]	—	50 ± 2
Adrenals (2)	61 ± 13	20 ± 3	−67[b]	—	—
Kidneys (2)	8.6 ± 0.7	6.3 ± 1.2	−27[b]	—	71 ± 18
Liver	9.0 ± 0.7	6.6 ± 0.7	−27[b]	—	21 ± 2
Salivary gland	—	—	—	—	31 ± 2
Brain	0.0	0.0	—	—	>1
Serum	—	—		—	8.5 ± 3

[a] Values are means ± SEM. Values in parentheses indicate data as percent of body weight. [b] Significant difference at p 0.05 level. Tissues were from the control and 50 mg/kg aminoguanidine-treated groups of rats in Experiment II.

significant increase of appetite in children[11–13] and in chronically underweight adults[14–16], and this effect has been confirmed in clinical trials[17,18]. A number of antidepressant drugs, notably amitriptyline[19], have also been observed to increase appetite and weight. The average gain in weight with these drugs was 2.5 to 5 kg and subjects noted particularly a craving for carbohydrate foods.

The present results suggest that another type of compound, aminoguanidine, may have similar effects in humans. However, because the effect of tumor, cytotoxic drugs and psychological factors could not be assessed, further studies were done in rats, where it was possible to use large numbers of animals, standard experimental conditions, and daily monitoring of body weight, daily food consumption.

Increased food consumption and weight gain were evident in 2 separate experiments. About 30% of the food consumed was converted to body tissue in both the control and treated rats, and this increase appeared to be generally distributed in body tissue rather than in increased fat deposition. Aminoguanidine accumulated in kidney and other tissues but not in brain as has been observed in earlier studies[5]. As in humans[2], the drug inhibited DAO activity in various tissues, especially in those tissues which normally contained high levels of this enzyme.

The mechanism for the increase in food consumption is unknown. The inability to detect aminoguanidine in brain suggests that either the drug is capable of acting centrally in trace amounts or that it acts peripherally in some manner. Earlier studies have shown that aminoguanidine potentiates ganglionic transmission and enhances salivation and gastric secretion due to cholinergic stimulation[20]. Whether this is a possible mechanism of the increased appetite production requires further study. Aminoguanidine is a known derivatizing reagent for sugars, and another possible mechanism is through reduction in blood or tissue glucose. It was, in fact, tested for this purpose 50 years ago as a possible antidiabetic agent, but it had no apparent effect[21].

Zusammenfassung. Nach Behandlung mit dem Diaminoxidase (DAO)-Hemmer Aminoguanidin wurden Appetitzunahme, vermehrte Nahrungsaufnahme und Gewichtszunahme sowohl bei einem Krebspatienten als auch bei Ratten beobachtet, wobei der Mechanismus des Effektes unklar blieb. Die Anreicherung der Droge sowie die Hemmung der DAO in den Geweben wurde gemessen.

S. BAYLIN, ZDENKA HORAKOVA and M. A. BEAVEN[22]

Pulmonary Branch and Hypertension- EndocrineBranch, National Heart and Lung Institute, National Institutes of Health, Building 10, Room 5N107, Bethesda (Maryland 20014, USA), 14 October 1974.

[11] A. F. LAVENSTEIN, E. P. DACANEY, L. LASAGNA and T. E. VAN METRE, J. Am. med. Ass. *180*, 912 (1962).
[12] S. S. BERGEN, JR., Am. J. Dis. Child. *108*, 270 (1964).
[13] A. DRASH, J. ELLIOTT, H. LANGS, A. F. LAVENSTEIN and R. E. COOKE, Clin. Pharmac. Ther. *7*, 340 (1966).
[14] S. VALIENTE, G. BAHAMONTES and A. TORO, Bol. Hosp. S. Juan, Santiago *14*, 342 (1967).
[15] F. FRANCINI, J. C. SANTANA and J. KITROSEN, Orientación Méd. *795*, 126 (1968).
[16] P. MAINGUET, Practitioner *208*, 797 (1972).
[17] R. E. NOBLE, J. Am. med. Ass. *209*, 2054 (1969).
[18] J. N. STIEL, G. W. LIDDLE and W. W. LACY, Metabolism *19*, 192 (1970).
[19] E. S. PAYKEL, P. S. MUELTER and P. M. DE LA VERGNE, Br. J. Psychiat. *123*, 501 (1973).
[20] W. B. SEVERS, J. W. GORDON, M. A. BEAVEN and S. JACOBSEN, Pharmacology *3*, 201 (1970).
[21] G. A. ALLES, J. Pharm. exp. Ther. *28*, 251 (1926).
[22] A preliminary account of these observations was presented at the FASEB Meeting, Altantic City, April 1971; Fedn. Proc. *30*, 233 (1971).