A comparison of variance estimates[10] between 7 control and 9 isethionate experiments gives an F value = 15.2 with $p < 0.001$. We have therefore quantified the effect by comparing the percentage change in activity in sample 9 with that in sample 7 in both control and isethionate experiments, using the non-parametric Mann-Whitney test[10] (in the isethionate experiments sample 7 immediately preceded the beginning of isethionate CSF perfusion). The median increase in GABA efflux calculated in this way was 27% ($p < 0.01$). This can be compared with the effect of superfusion of the cuneate nucleus for a 10-min period with CSF with an elevated (40 mM) potassium content (figure,C) which produces a mean increase in GABA efflux of 30%[11]. The possibility that the isethionate-induced GABA efflux seen in the experiments reported here could have been due to some discrepancy in the potassium content of normal and isethionate CSF has been discounted. Potassium contents were measured by flame-emission spectrometry and found to be identical.

It is unlikely that the increase in GABA efflux is a specific pharmacological effect of the isethionate molecule. A similar effect has been observed in 2 preliminary experiments using methylsulphate instead of isethionate as a substitute for chloride. It is therefore possible that any impermeant substitute for chloride might have the same effect. This brings into question the usefulness of impermeant anions as replacement for extracellular chloride in electrophysiological studies in the central nervous system.

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2 N. B. supported by grants from CAPES and FAPESP, Brazil, J. A. A. supported by grants from CNPq and FAPESP, Brazil.
3 B. Katz and R. Miledi, J. Physiol. *168*, 389 (1963).
4 J. L. Barker and R. A. Nicoll, J. Physiol. *228*, 259 (1973).
5 N. Davidson and H. K. L. Simpson, Experientia *32*, 348 (1976).
6 S. Nishi, S. Minota and A. G. Karczmar, Neuropharmacology *13*, 215 (1974).
7 N. Davidson and C. A. P. Southwick, J. Physiol. *219*, 689 (1971).
8 J. A. Assumpção, N. Bernardi, C. G. Dacke and N. Davidson, J. Physiol. *263*, 231 (1976).
9 J. K. Merlis, Am. J. Physiol. *131*, 67 (1940).
10 R. Meddis, Statistical Handbook for Non-statisticians. McGraw-Hill, London 1975.
11 J. A. Assumpção, N. Bernardi, C. G. Dacke and N. Davidson, Br. J. Pharmac. *59*, 4889 (1977).

Pancreatic polypeptide: A possible role in the regulation of food intake in the mouse. Hypothesis

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Summary. Pancreatic polypeptide (PP) is a recently identified hormone produced by pancreatic endocrine cells. The islets of genetically obese mice (ob/ob, C57 BL/6J), which are suspected to lack a circulating satiety factor, contain relatively few of the PP-producing cells. Administration of bovine pancreatic polypeptide (bPP) reduces food intake and suppresses body weight gain in the hyperphagic obese mice. It is postulated that PP participates in the regulation of food intake in a manner as yet undefined.

Pancreatic polypeptide (PP) is a new hormone, first isolated from avian pancreas[4] and later identified in several mammalian species, including man[5,6]. In the pancreas, this hormone is stored in both insular and extrainsular cells, which can be distinguished from insulin-, glucagon-, and somatostatin-containing cells by immunohistochemical criteria[7-11]. In rats and mice, the PP-containing cells are particularly numerous in the islets located in the cephalic part of the pancreas, where they rank second in number after insulin-containing cells[12]. Although exogenous PP affects several parameters of hepatic and gastro-intestinal function[5,13,14], its physiological significance remains unknown. Because the plasma concentration of PP increases after feeding[15-18], we have begun an investigation on the possible role of PP in the regulation of food intake. The present part of the study was performed in hereditarily obese mice, in which hyperphagia is tentatively attributed to the lack of a circulating satiety factor[19].

Materials and methods. 18 obese (ob/ob) and 6 age-matched lean (+/+) mice of the C57 BL/6J strain were purchased

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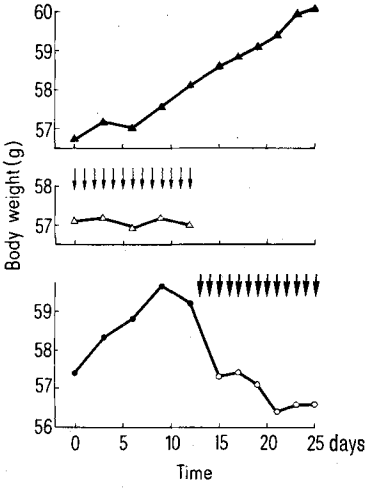
4 J. R. Kimmel, H. G. Pollock and R. L. Hazelwood, Endocrinology *83*, 1323 (1968).
5 T. M. Lin and R. E. Chance, Gastroenterology *62*, 852 (1972).
6 T. M. Lin and R. E. Chance, Gastroenterology *67*, 737 (1974).
7 L. I. Larsson, F. Sundler, R. Hakanson, H. G. Pollock and J. R. Kimmel, Histochemistry *42*, 377 (1974).
8 L. I. Larsson, F. Sundler and R. Hakanson, Cell Tissue Res. *156*, 167 (1975).
9 L. I. Larsson, F. Sundler and R. Hakanson, Diabetologia *12*, 211 (1976).
10 D. Baetens, C. Rufener and L. Orci, Experientia *32*, 785 (1976).
11 C. Rufener, D. Baetens and L. Orci, Experientia *32*, 919 (1976).
12 L. Orci, D. Baetens, M. Ravazzola, Y. Stefan and F. Malaisse-Lagae, C. r. Acad. Sci. (Paris) *283*, 1213 (1976).
13 R. L. Hazelwood, S. D. Turner, J. R. Kimmel and H. Pollock, Endocrinology *21*, 485 (1973).
14 T. M. Lin, R. E. Chance and D. Evans, Gastroenterology *64*, 865 (1973).
15 D. R. Langslow, J. R. Kimmel and H. G. Pollock, Endocrinology *93*, 558 (1973).
16 J. C. Floyd, Jr., and S. S. Fajans, Diabetes *25*, 37 (1976).
17 T. E. Adrian, S. R. Bloom, M. G. Bryant, J. M. Polak, P. Heitz and A. J. Barnes, Gut *17*, 940 (1976).
18 T. E. Adrian, S. R. Bloom, H. S. Besterman, A. J. Barnes, T. J. C. Cooke, R. C. G. Russel and R. G. Faber, Lancet *1*, 161 (1977).
19 D. L. Coleman, Diabetologia *9*, 294 (1973).

Mice	Treatment	Change in b.wt (mg/day)	Food intake (g/animal · day)
+/+	Nil	+ 36 ± 12 (18)	3.09 ± 0.06 (20)
ob/ob	Nil	+ 144 ± 53 (6)	6.23 ± 0.26 (7)
ob/ob	Saline	+ 113 ± 16 (18)	5.72 ± 0.07 (20)
ob/ob	bPP (10 μg/kg day)	− 6 ± 17 (6)	5.07 ± 0.24 (6)
ob/ob	bPP (100 μg/kg day)	− 192 ± 78 (6)	5.03 ± 0.12 (6)

Mean values (± SEM) for the change in body weight and for food intake are indicated together with the number of individual observations. Each individual value for weight gain refers to a single mouse examined over 1 (lines 2, 4, 5) or 3 (lines 1 and 3) periods of 12 to 13 days each. Each individual value for food intake refers to a group of 6 mice examined over successive periods of 2 days each.

from the Jackson Laboratory (Bar Harbor, Maine) and, when aged $3^1/_2$ months, housed in groups of 6 animals each in cages with free access to tap water and a standard pellet diet (A.04, UAR, Villemoisson s/Orge, France). Bovine pancreatic polypeptide (bPP; lot 615-D65-295, a generous gift of Dr R. E. Chance, Lilly Research Laboratory, Indianapolis, Indiana, USA) was solubilized in 0.3% (v/v) acetic acid and, from such a stock solution, appropriately diluted in saline immediately prior to injection into the animals.

The obese mice were randomly distributed among 3 groups. The first group served as control, the animals receiving 2 daily i.p. injections (at 09.00 h and 18.00 h) of saline, throughout the experimental period. The obese mice in the second group were injected, according to the same schedule, with a low dose of bPP (2 × 5 μg/kg b.wt per day) administered from the onset of the experimental period. In the third group, the obese mice remained untreated during the first 12 days and were then injected with a higher dose of bPP (2 × 50 μg/kg b. wt per day). The body weight of each animal was measured between 08.00 and 09.00 h every second or third day. The total amount of food consumed by the 6 mice present in each case was measured over successive periods of 48 h each. For the 2 groups of bPP-treated mice, only those results recorded during the 12 first days of treatment are here presented. Indeed, for reasons presently under investigation, the effect of bPP upon weight gain and food intake tended to fade out after 2 weeks of treatment.



Mean values for b. wt in ob/ob mice (n = 6 in each case) injected i.p. with saline (top panel), or with bPP at a daily dose of either 10 (fine arrows; middle panel) or 100 (thick arrows; lower panel) μg/kg b. wt.

Results. Obese mice gained significantly more weight than the lean mice ($p < 0.01$). Injection of saline slightly reduced the daily weight gain of the obese mice. Over the 12 days of treatment considered, administration of bPP invariably resulted in a significant reduction ($p < 0.001$) in daily weight gain, this effect being more marked ($p < 0.05$) at the high than low dose of the hormone (table). Thus, at the low dose of bPP, all the animals, instead of gaining weight, remained at their initial weight throughout the period of treatment. At the high dose of bPP, a reduction in body weight was noticed in every animal (figure).

Untreated ob/ob animals ingested twice as much food (g/day per animal) as normal mice. Injection of saline to the obese mice apparently resulted in a significant ($p < 0.025$) reduction in food intake. When treated with bPP, in either low or high dose, the animals ingested less food than the saline-injected obese mice. The difference between bPP-treated and saline-injected animals was significant, whether judged from the data shown in the table ($p < 0.005$ or less) or from comparison of data collected in each group over the same period of time ($p = 0.05$ at the low bPP dosis; $p < 0.001$ at the high bPP regimen). Although the relative magnitude of the bPP-induced changes in food intake appears rather modest, comparison of the differences in both weight gain and food intake between saline-injected and bPP-treated mice indicated that each g reduction in daily food intake, as provoked by bPP, resulted in a 0.3 ± 0.1 g daily loss in body weight, a value close to that expected from the caloric value of the chow (ca. 2.9 cal/g).

Incidentally, because of the short half-life of exogenous PP in body fluids, its effect upon food intake, as revealed by the present data, may be the result of only an intermittent inhibitory action in the mice having free access to food throughout the experimental period.

Discussion. One of the obesity-diabetes syndromes in C57 BL/6J mice of Bar Harbor is caused by the gene obese (ob) located in chromosome 6[20]. This syndrome is characterized by hyperphagia, obesity, hyperglycemia and hyperinsulinemia. The exact role of hyperphagia in the complex perturbations observed in this syndrome is as yet unclear, but it is a constant phenomenon. At variance with normal mice, ob/ob mice do not exhibit diurnal variations of food intake but eat constantly a large amount of food throughout day and night[21]. Parabiosis experiments carried out by Coleman[19] suggest that ob/ob mice seem to possess a functioning satiety centre (sensitive to a circulating factor produced by the lean mouse) but they are apparently unable to produce sufficient satiety factor to regulate their own food consumption. In the course of studying relative changes in islet cell populations in Bar Harbor mice, we were struck by the fact that in the hyperplastic islets of obese animals, 2 islet cell types are particularly reduced in their relative abundance, the somatostatin-containing and the PP-containing cells[22]. In our view, PP represents a better

20 D. L. Coleman and K. P. Hummel, Diabetologia *9*, 287 (1973).
21 C. J. Bailey, T. W. Atkins, M. J. Conner, C. G. Manley and A. J. Matty, Hormone Res. *6*, 380 (1975).
22 D. Baetens, Y. Stefan, M. Ravazzola, F. Malaisse-Lagae, D. L. Coleman and L. Orci, submitted for publication (1977).
23 J. M. Polak, S. R. Bloom and T. E. Adrian, Lancet *1*, 328 (1976).

candidate than somatostatin as a satiety factor, since PP is detected in the systemic circulation[15,17,23] (whereas such may not be the case for somatostatin) and since the plasma concentration of PP indeed increases in response to food intake[15–18].

The present work reveals that exogenous PP, when administered to the hyperphagic ob/ob mice, reduces food intake and causes a dose-related decrease in body weight gain. These results are compatible with the hypothesis that PP participates in the regulation of food intake. The demonstration that hyperphagia in obese mice is indeed associated to decreased levels of circulating pancreatic polypeptide awaits the availability of a method of measurement of PP in murine plasma.

Adenosine 3′,5′-monophosphate in snail (Helix pomatia) nervous system: Analysis of dopamine receptors

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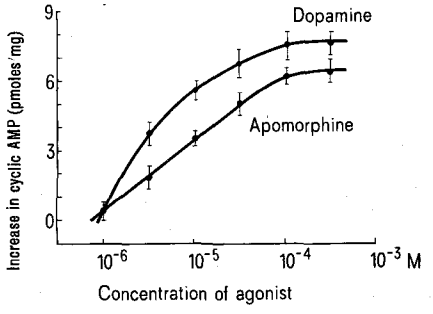
Summary. The effect of dopamine on snail (Helix pomatia) nervous tissue adenosine 3′, 5′-monophosphate content was examined. The results show support for the idea that the dopamine receptors in the snail nervous system involve adenylate cyclase. It is suggested that these are the dopamine excitatory receptors rather than the inhibitory ones.

Considerable evidence has accumulated implicating dopamine as a neurotransmitter in the snail brain. It is localized in a small proportion of neuron perikarya, their axons and presumed presynaptic endings[2–4], and all the data from experiments on the release of dopamine from a single neuron containing the amine[5], the occurrence of dopamine receptors on certain gastropod neurons[5–7], the presence of dopamine within synaptic-type vesicles[8] and the demonstration of a high affinity uptake process for dopamine[9] tend to substantiate the hypothesis that the amine is involved in some specific aspect of function in the snail CNS. With regard to the nature of the dopamine receptor in the gastropods, a number of electrophysiological studies have been carried out suggesting that at least 2, and probably more, types of dopamine receptor exist in their nervous tissues[10]. Moreover, an adenylate cyclase, which is activated by low concentrations of dopamine[11], has recently been demonstrated to occur in the gastropod nervous system, and this, with other data[12,13], suggests that dopamine receptors of gastropod nervous tissues may be identical with the dopamine-binding moiety of dopamine-sensitive adenylate cyclase, and that the effects of dopamine on the central nervous system may be due to dopamine-induced increases in adenosine 3′, 5′-monophosphate (cyclic AMP) in the post-synaptic cells. Strong evidence exists to support the opinion that cyclic AMP mediates the effect of dopamine in the mammalian superior cervical ganglion, retina and caudate nucleus[14] and also in the thoracic ganglia of the cockroach[15]. In view of the impressive data supporting a transmitter role for dopamine in the gastropod central nervous system, I examined more closely the effects of dopamine on cyclic AMP content with the aim of characterizing the receptors.

Material and methods. Active snails caught daily in the surrounding woods of Göttingen were used. Adenylate cyclase activity was measured in homogenates of tissue according to the method of Kebabian et al.[16] with some modifications. The standard assay system (final volume 500 µl) contained (in mmoles/litre): tris (hydroxymethyl) aminomethane-maleate 90; ATP, 0.5; Mg SO_4, 2.0; ascorbate, 0.5; theophylline, 5; EGTA, 0.2; plus test substances as indicated. The reaction was initiated by the addition of ATP. Incubation was for periods of 15 min in a



Increase in cyclic AMP in homogenates of snail central ganglia as a function of the concentration of dopamine and apomorphine. The data represent the mean ± SEM for 30 replicate samples in the case of dopamine, and 20 replicate samples in the instance of apomorphine. In the absence of added agonist, the cyclic AMP content was 2.78 ± 0.33 pmoles/mg wet weight of tissue (± SEM for 30 experiments).

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2 E. Dahl, B. Falck, C. von Meklenburg, H. Myhrberg and E. Rosengren, Z. Zellforsch. *71*, 489 (1966).
3 N. Osborne and G. A. Cottrell, Z. Zellforsch. *112*, 15 (1971).
4 N. N. Osborne, E. Priggemeier and V. Neuhoff, Brain Res. *90*, 261 (1975).
5 M. S. Berry and G. A. Cottrell, Nature New Biology *242*, 250 (1973).
6 P. Ascher, J. Physiol. *225*, 173 (1972).
7 R. J. Walker, G. N. Woodruff, B. Glaizner, C. B. Sedden and G. A. Kerkut, Comp. Biochem. Physiol. *24*, 455 (1968).
8 V. W. Pentreath and G. A. Cottrell, Experientia *30*, 293 (1974).
9 N. N. Osborne, L. Hiripi and V. Neuhoff, Biochem. Pharmac. *24*, 2141 (1975).
10 P. Ascher and J. S. Kehoe, in: Handbook of Psychopharmacology, vol. 4, p. 265. Ed. L. L. Iversen, S. Iversen and S. Snyder, 1975.
11 H. Cedar and J. H. Schwartz, J. gen. Physiol. *60*, 570 (1972).
12 H. Cedar, E. R. Kandel and J. H. Schwartz gen. Physiol. *60*, 558 (1972).
13 S. N. Treistan and I. B. Levitan, Nature *261*, 62 (1976).
14 D. H. York, Brain Res. *37*, 91 (1972).
15 J. A. Nathanson and P. Greengard, Science *180*, 308 (1973).
16 J. W. Kebabian, G. L. Petzold and P. Greengard, Proc. nat. Acad. Sci. USA *69*, 2145 (1972).