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Pancreatic and Intestinal Digestive Enzymes in Post-Weanling Rats With Hypothalamic Obesity[1]

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BERNARDIS, L. L., P. C. LEE, S. BROOKS AND E. LEBENTHAL. *Pancreatic and intestinal digestive enzymes in post-weanling rats with hypothalamic obesity.* PHARMACOL BIOCHEM BEHAV 22(4) 589–598, 1985.—Male Sprague-Dawley rats received bilateral electrolytic lesions in the ventromedial hypothalamic nuclei (VMNL rats) at the age of 31 days; sham-lesioned rats served as controls. For 28 post-operative days all animals self-selected from three synthetic diets, each high in carbohydrate, fat and protein, respectively. Following this, half of the VMNL rats and half of the controls were switched to lab chow for 14 days. Body weights were comparable among the groups, but linear growth was greatly reduced and body fat (Lee Index) was elevated in VMNL rats, irrespective of diet. In the sham-lesioned controls, the synthetic diets reduced most parameters of exocrine pancreatic activity. In VMNL rats, in contrast, pancreatic parameters were unaffected by the synthetic diet. The data suggest that VMN lesions disinhibit the exocrine pancreas. In contrast, most parameters of intestinal activity were not influenced by VMN lesions.

Weanling rats Ventromedial hypothalamic nucleus Linear growth Obesity Synthetic diets Exocrine pancreas Intestinal enzyme activity

STIMULATION of exocrine pancreatic secretion is subserved by cephalic, gastric and intestinal mechanisms [21, 26, 34]. Although a cephalic phase of gastric secretion has been recognized [25], a cephalic phase of the exocrine pancreas has been thought to be of less importance [32]. Some authors have attributed this phase of pancreatic secretion to a direct vagal effect [40], whereas others have suggested that the response is mediated by vagally released gastrin from the pyloric gland area [37]. Whatever the mechanism, it is agreed that the vagus is involved in this phenomenon.

The vagus has also been shown to play a predominant role in the neuroendocrine-metabolic-behavioral alterations that follow experimental destruction of the ventromedial hypothalamic area (VMH): obesity produced by VMH lesions in mature rats can be eliminated by subdiaphragmatic vagotomy [35]. Although a "cephalic" hypothesis [36] has proposed that changes observed after VMH destruction are due to an exaggerated response to autonomic and endocrine reflexes to the sensory contact with food, subsequent data have put these findings into a new perspective. For instance, King *et al.* [24] have reported that although fasting hyperinsulinemia in VMH rats is under vagal control, vagotomy does not prevent the development of obesity.

In a previous communication [27] we had reported that weanling rats with lesions in the ventromedial hypothalamic nuclei (VMNL rats) that became obese in the face of normophagia and normal body weight [5] exhibit reduced pancreatic weight that was evident two weeks after lesion production and became more pronounced during the following three weeks. Furthermore, both pancreatic protein and DNA content failed to increase over time in VMNL rats as they did in sham-operated control rats. However, pancreatic content of amylase, lipase and trypsinogen were comparable in VMNL rats and controls, as were concentrations of amylase and lipase. Pancreatic trypsinogen concentration, on the other hand, was elevated in VMNL rats throughout all observation periods [27].

Pancreatic enzyme contents and secretion are not only controlled by mechanisms of cephalic origin but are also influenced by adaptation, depending on composition and amount of substrates in the ingesta [11, 14, 29]. In view of the fact that mature [41] and weanling [6,7] VMNL rats select different amounts of macronutrients than controls, and in view of the Powley [36] concept of a vagal role of VMH obesity we designed the present experiment to investigate the effects of VMN lesions in weanling rats on pancreatic

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TABLE 1

ACTUAL MACRONUTRIENT COMPOSITION AND CALORIC DENSITY OF THREE DIETS AND LAB CHOW USED IN THE PRESENT STUDY

Dietary Constitutent*	High Carbohydrate Diet (HCD)‡	High-Fat Diet (HFD)‡	High-Protein Diet (HPD)‡	Lab Chow§
Vitamin-free Casein	18.00	18.00	40.73	
DL-methionine	0.3	0.3	0.3	
Vitamin-Diet Fortification Mix	1.0	1.0	1.0	
Salt Mixture USP ×111	4.0	4.0	4.0	
Starch	23.8	16.0	32.7	
Sucrose	47.6	32.7	16.0	
Corn Oil	5.0	15.1	5.0	
Choline Chloride	0.1	0.1	0.1	
Cellulose	0.2	12.8	0.17	
Carbohydrate†	75.7	59.6	51.6	52.0
Fat†	5.3	18.4	5.2	5.5
Protein†	19.0	22.0	43.2	22.5

*Percent by weight of all constituents (Custom-made by ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH 44128); †percent by weight of all three macronutrients; ‡caloric density of all three diets: 16.12 kj (4.03 kcal)/g; §caloric density of lab chow: 15.60 kj (3.9 kcal)/g. Other nutritional data: fiber 4.0%, Ash 5.5%, Moisture 10.0% (Charles River Rat Mouse Hamster Formula, Country Foods, Division of Agway, Hauppauge LI, NY 11787).

and intestinal enzymes. The weanling rat also appeared to us a good experimental model because it develops obesity in the presence of normal food intake and body weight gains. Male rats were chosen because all of our previous neuroendocrine-metabolic studies had been performed in male weanling rats [5,19].

METHOD

Weanling male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) were accommodated in individual cages in a light cycle (L:D 12:12, lights on at 0600 hr) and temperature-controlled (23°C) room and fed Charles River Rat Mouse Hamster Formula (Table 1) ad lib. At the age of 31 days the animals were anesthetized with sodium hexobarbital and received bilateral electrolytic lesions in the ventromedial hypothalamic area. A direct anodal current of 1.5 mAmp flowed for 12 seconds from the bared (0.25 mm) tip of a spar varnish-coated stainless steel electrode of 0.37 mm diameter. Sham-lesioned animals (electrode inserted dorsal to the ventromedial nucleus (VMN) but without current flow) served as controls. Details of the operation have been described previously [6]. At the time of surgery all animals were also weighed and their nose-tail and naso-anal lengths determined for the assessment of linear growth and the calculation of the Lee Index [3,44]. The timing of these and other experimental manipulations is summarized in Table 2. The animals were exposed to two dietary periods, henceforth referred to as Period 1 and Period 2. During Period 1, which started following the hypothalamic operation and lasted 28 days, all rats received three equicaloric synthetic diets that were selected because of their content of sucrose (Table 1). The diets were supplied in color-coded pellets and were presented simultaneously in stainless steel hoppers (90×75 mm, Unifab Corporation, Kalamazoo, MI, No. US-161-A) that were suspended from the rear of the cage and provided with cardboard dividers. This created three equal spaces to accommodate the diets. The position of the diets in each hopper was changed daily in random fashion. Food intake was weighed throughout the experiment in three-day blocks and spillage was considered in the weighings. Since each diet contained different amounts of macronutrients (Table 1), the dietary data are presented in terms of macronutrient intake/day from all three diets rather than in terms of amounts of each diet selected [6].

At the end of Period 1 (28 days after the operation; age 59 days) all rats were anesthetized with ether for the precise determination of body weight and linear growth and the computation of the Lee Index and were then subdivided into four groups.

During Period 2 (14 days duration) one group of VMNL rats (Group 1) and one control group (Group 2) continued to receive the three synthetic diets, whereas a second VMNL group (Group 3) and controls (Group 4) were switched from the synthetic diets to lab chow (Table 1). At the end of this period (42 days post-op; age 73 days), all animals were again weighed and measured as above and killed on the following day by decapitation.

The brains were removed and processed as previously described for the histological analysis of the lesions [4].

The whole intestine from the pyloric to the ileo-cecal region was removed and trimmed of fat and mesentery. The length of the intestine was measured during free suspension with a 5 g weight attached to the end. A length of 15 cm was removed from the proximal end representing the duodenum. The segment was split and the intestinal content removed by gently wiping with tissue paper. The mucosa was scraped and weighed separately and the mucosal preparation from each segment was separately homogenized with a Potter-Elvehjem homogenizer using a teflon pestle with a vessel immersed in crushed ice. Homogenates were used for the determination of DNA, protein, lactase, maltase, sucrase and enterokinase.

Deoxyribonucleic acid was first precipitated with a 0.5 N

TABLE 2

TIME-EVENT FLOW CHART

Manipulation	Age (days)	Days Post-Operatively	Duration of Diet Period (days)
Operation, sham-operation, Weigh and measure rats, Feed 3 equicaloric diets	31	0	
Weigh and measure, Gr. 1 and 2 continue on 3 diets, Gr. 3 and 4 switched to lab chow	59	28	28
Weigh and measure	73	42	14
Kill	74	43	

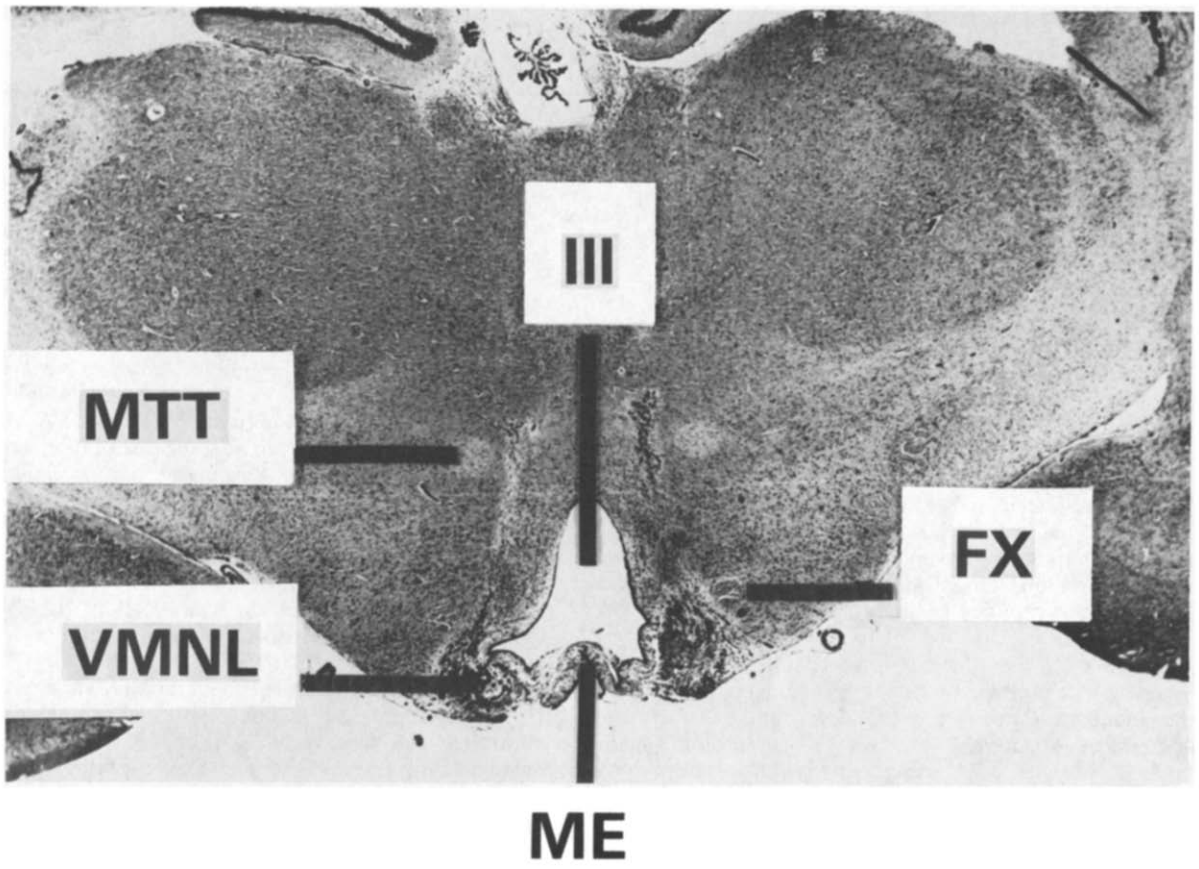

FIG. 1. Coronal section through the hypothalamus at the largest cross section of the lesions of a rat representative of Groups 1 and 3. Abbreviations: III: third ventricle; FX: fornix; ME: median eminence; VMNL: lesions in the ventromedial nuclei; MTT: mamillothalamic tract. (Cresyl Violet.)

TABLE 3
SOMATIC PARAMETERS

Group	1	2	3	4							
N	4	4	4	6							
Operation	VMNL**	CON	VMNL	CON							
						Effect of		Tukey's Test $p<$			
Diet	Synth	Synth	Chow	Chow	Diet*	Lesion*	Interaction*	1–2	1–3	3–4	2–4
Δ Body Weight (g)***	6.06§	5.32	6.79	5.34		9.55†					
First Period (28 days)	±0.57	0.37	0.59	0.14	N.S.	0.01††	N.S.	N.S.	N.S.	0.05	N.S.
Δ Body Weight (g)	3.36	0.54	1.39	2.92			6.14				
Second Period (14 days)	±0.73	0.37	1.76	0.72	N.S.	N.S.	0.05	N.S.	N.S.	N.S.	N.S.
Δ Body Length (mm)	2.20	4.31	2.55	4.42		94.67					
First Period (28 days)	±0.42	0.25	0.16	0.09	N.S.	0.001	N.S.	0.01	N.S.	0.01	N.S.
Δ Body Length (mm)	0.93	1.78	0.79	1.66		12.53					
Second Period (14 days)	±0.21	0.17	0.07	0.21	N.S.	0.01	N.S.	0.01	N.S.	0.01	N.S.
Lee Index§§	362.8	301.8	366.3	299.5		686.85					
First Period (28 days)	±1.96	2.64	3.51	2.55	N.S.	0.001	N.S.	0.01	N.S.	0.01	N.S.
Lee Index	373.5	296.8	366.3	299.7		282.68					
Second Period (14 days)	±6.48	2.77	6.56	3.32	N.S.	0.001	N.S.	0.01	N.S.	0.01	N.S.

*ANOVA data, F=(1,14).
**VMNL=rats with ventromedial lesions, CON=sham-operated controls.
***Body weight and length changes during Period 1 and 2, divided by number of days per period.
§Mean ± SEM.
§§Lee Index=cube root of body weight (g)/naso-anal length (mm)·10^4 (Bernardis, 1970).
†F value.
‡P value of ANOVA.

perchloric acid, hydrolyzed, and the deoxyribonucleotide residue measured by the colorimetric reaction with a diphenylamine reagent according to Burton [10], using highly polymerized calf thymus DNA as the standard. A modified procedure was adapted to minimize the interference by sialic acid according to Croft and Lubran [15].

Protein was determined using the method of Lowry *et al.* [30]. Maltase, lactase and sucrase were assayed by the method of Dahlquist [16], using maltose, lactose and sucrose as the corresponding substrates. Disaccharidase activity was expressed as micromoles of disaccharides hydrolyzed per min/g protein.

Enterokinase was assayed in two steps. The mucosal homogenate was preincubated with trypsinogen and aliquots of the incubation mixture were determined for trypsin formed. Trypsin activity was assayed according to the method of Erlanger [17], using benzoyl-DL-arginine-p-nitroanilide as the substrate. Activity was expressed as micromoles of substrate hydrolyzed per g protein.

Pancreata were removed, trimmed of fat and mesenteries and processed for the determination of protein, DNA, trypsinogen, lipase and amylase.

Pancreatic tissues were minced with sharp scissors to facilitate further homogenization in distilled water (1 mg/100 μl) at 4°C with a Potter-Elvehjem homogenizer and a teflon pestle. Enzyme activities were determined from fresh homogenate whenever possible. Homogenate stored at −20°C lost negligible enzyme activity after a single thawing.

Trypsinogen was first activated with partially purified rat mucosal enterokinase at a constant ratio of enterokinase to homogenate protein for 45 min at 25°C, conditions that yielded optimal and reproducible activation of this zymogen in our laboratory. Trypsin activity was then measured from the hydrolysis of p-nitro-aniline from the substrate benzoyl-DL-arginine-p-nitroaniline (BAPNA) at pH 8.2 and 25°C. Units are expressed as nanomoles of BAPNA hydrolyzed per min per mg protein. Lipase activity was determined by potentiometric titration (at a constant pH of 8.0) of ionized fatty acids liberated from an olive oil emulsion [43]. Units are expressed as micromoles of NaOH required to neutralize the free fatty acid liberated per min per mg protein. Amylase was determined by the saccharogenic method [42], using soluble starch as the substrate. Units are expressed as micromoles of maltose liberated per min per mg protein.

Protein was determined by the Lowry [30] technique, using bovine serum albumin fraction V as the standard. Deoxyribonucleic acid was determined as above.

The data were analyzed using Analysis of Variance (ANOVA) and Tukey's test.

TABLE 4

MACRONUTRIENT INTAKE (g/day)

Group	1	2	3	4							
N	4	4	4	6							
Operation	VMNL**	CON	VMNL	CON							
						Effect of		Tukey's Test $p <$			
Diet	Synth	Synth	Chow	Chow	Diet*	Lesion*	Interaction*	1–2	1–3	3–4	2–4
Carbohydrate Intake	12.28§	9.07	13.15	8.96		53.48†					
Period 1	±0.57	0.31	0.87	0.43	N.S.	0.001††	N.S.	0.01	N.S.	0.01	N.S.
(28 days)	(65.3)	(66.2)	(65.1)	(69.5)							
Carbohydrate Intake	9.53	8.52	10.19	13.45	5.83						
Second Period	±0.59	0.48	2.09	0.92	0.05	N.S.	N.S.	N.S.	N.S.	N.S.	0.01
(14 days)	(61.1)	(63.0)	(68.6)	(73.1)							
Fat Intake	2.30	1.61	2.36	1.58		17.41					
Period 1	±0.40	0.10	0.13	0.12	N.S.	0.001	N.S.	N.S.	N.S.	0.01	N.S.
(28 days)	(12.2)	(11.7)	(11.6)	(11.4)							
Fat Intake	2.02	1.25	1.65	1.57							
Period 2	±0.42	0.20	0.21	0.07	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
(14 days)	(12.9)	(9.2)	(11.1)	(8.5)							
Protein Intake	4.22	3.08	4.70	3.35		32.72					
Period 1	±0.34	0.22	0.24	0.19	N.S.	0.001	N.S.	0.01	N.S.	0.01	N.S.
(28 days)	(22.5)	(22.5)	(23.3)	(24.1)							
Protein Intake	3.79	3.75	3.02	3.39							
Period 2	±0.16	0.27	0.66	0.23	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
(14 days)	(24.6)	(27.8)	(20.3)	(18.4)							
Total Macronutrient Intake	18.79	13.71	20.21	13.90		56.75					
Period 1	±1.26	0.62	1.19	0.43	N.S.	0.001	N.S.	0.01	N.S.	0.01	N.S.
(28 days)											
Total Macronutrient Intake	15.65	13.53	14.86	18.41							
Period 2	±0.91	0.81	2.74	1.20	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.05
(14 days)											

For abbreviations, see Table 3.

Percent total intake associated with each macronutrient in brackets ( ).

RESULTS

Lesion Localization

Figure 1 shows that the principal area of destruction in the hypothalamus of a representative rat is located in the ventromedial hypothalamic nuclei and that neither median eminence nor lateral hypothalamic area were injured.

Somatic Parameters

Table 3 indicates that at the end of Period 1 (during which all rats were fed synthetic diets) Group 3 (VMNL rats) had gained significantly more weight than Group 4 (controls). However, there was no difference in body weight gain between Group 3 and Group 1, the latter also being a VMNL group. Moreover, at the end of Period 2 (during which Groups 3 and 4 had been switched to lab chow) all groups of rats showed similar weight gains, i.e., there was neither a lesion nor a diet effect.

Table 3 also shows that both VMNL groups exhibited a significant linear growth retardation during Periods 1 and 2. However, there was no diet effect. In contrast to body weight, the Lee Index, a non-invasive measure of obesity [44] was dramatically increased in both groups of VMNL rats during both Periods 1 and 2. As with linear growth, there was no diet effect (Table 3).

Macronutrient Intake

During Period 1, mean intake from all three macronutrients was greater in VMNL than control rats, i.e., there was a strong lesion effect. However, during Period 2 (after Groups 3 and 4 had been switched over to lab chow), macronutrient intake was similar in all groups except for the synthetic diet-fed controls (Group 2) which ate less carbohydrate than chow-fed controls (Group 4).

Total macronutrient intake from carbohydrate, fat and protein combined was higher in VMNL rats than in controls only during Period 1, i.e., their hyperphagia during this period is a reflection of increased macronutrient intake from all sources (see above). As might be expected from the individual macronutrient intake during Period 2 (see above),

TABLE 5
PANCREATIC PARAMETERS

Group	1	2	3	4							
N	4	4	4	6							
Operation	VMNL**	CON	VMNL	CON							
Diet	Synth	Synth	Chow	Chow	Diet*	Effect of Lesion*	Interaction*	Tukey's Test $p<$ 1–2	1–3	3–4	2–4
Pancreas Weight (mg)	942.5§ ±84.2	372.5 ±57.3	1130.0 ±147.7	1401.7 ±59.6	59.62† 0.001††	N.S.	28.53 0.001	0.01	N.S.	0.05	0.01
Pancreas Protein (mg/Panc.)	115.9 ±15.3	21.4 ±4.1	130.5 ±8.5	187.2 ±14.3	62.04 0.001	— N.S.	43.54 0.001	0.01	N.S.	0.01	0.01
DNA (mg/Panc.)	6.00 0.37	2.66 0.19	7.12 0.90	9.55 0.70	46.42 0.001	— N.S.	24.09 0.001	0.01	N.S.	0.05	0.01
Total Act., Trypsin (U/Panc.)	6447.0 ±2046.1	276.7 ±226.6	6289.8 ±619.5	8303.0 ±775.3	16.68 0.01	4.64 0.05	18.09 0.001	0.01	N.S.	N.S.	0.01
Enz. Concentration (U/mg Prot).	51.95 ±13.13	10.15 ±6.52	48.93 ±7.00	44.35 ±2.03	5.99 0.05	13.25 0.01	8.53 0.05	0.01	N.S.	N.S.	0.01
Total Act., Amylase (U/Pancr.)	2848.5 ±1366.1	95.3 ±75.74	6324.8 ±609.8	26079.0 ±4395.2	26.12 0.01	8.70 0.01	15.25 0.01	N.S.	N.S.	0.01	0.01
Enz. Concentration (U/mg Protein)	23.73 ±9.72	3.55 ±2.26	46.88 ±9.82	137.13 ±15.07	49.99 0.001	9.99 0.01	24.82 0.001	N.S.	N.S.	0.01	0.01
Total Act., Lipase (U/Pancr.)	14321.3 ±5739.1	2736.0 ±3163.0	11539.0 ±2408.5	10606.3 ±1796.3	— N.S.	7.95 0.05	6.10 0.05	0.01	N.S.	N.S.	0.05
Enz. Concentration (U/mg Protein)	2.85 ±1.36	0.095 ±0.077	6.33 ±0.61	26.08 ±4.40	26.18 0.001	8.72 0.05	15.28 0.01	N.S.	N.S.	0.01	0.01

For abbreviations, see Table 3.

total intake was comparable in all groups. Thus, the hyperphagia of the VMNL rats during Period 1 had normalized. These data are shown in Table 4.

Pancreas

Table 5 shows that pancreatic weight, protein and DNA are higher in synthetic diet-fed VMNL rats than in synthetic diet-fed controls. Synthetic diet-fed controls also had lower values than chow-fed controls but no such effect was evident in the two VMNL groups.

Total activity and concentration of trypsinogen were higher in synthetic diet-fed VMNL rats than in synthetic diet-fed controls but there was no difference between synthetic diet-fed VMNL rats and chow-fed VMNL rats. Both total activity and concentration were greatly reduced in synthetic diet-fed controls below that of chow-fed controls, thus resembling the pattern of changes in pancreatic weight, protein and DNA (Table 5).

Total amylase activity and concentration were comparable in synthetic diet-fed VMNL rats and controls whereas chow-fed VMNL rats exhibited greatly reduced activities below that of chow-fed controls. Synthetic diet-fed controls showed dramatically lower total activity and concentration than chow-fed controls (Table 5).

The only instance in which the profiles of total activity and concentration diverged is that of pancreatic lipase; synthetic diet-fed VMNL rats had activities comparable to chow-fed VMNL rats but the synthetic diet-fed controls exhibited lower activity and enzyme concentration than synthetic diet-fed VMNL rats and chow-fed controls. However, this was significant only in the case of total activity (Table 5).

Small Intestine

Table 6 shows that mucosal weight, protein, DNA and total activity and concentration of sucrase were comparable among all groups with the exception of mucosal protein, in that synthetic diet-fed VMNL rats showed lower values than their controls. Total maltase activity was also similar among all groups but maltase concentration was greater in synthetic diet-fed than in chow-fed controls (Table 6).

Synthetic diet-fed VMNL rats had reduced total activity and concentration of enterokinase in comparison with their controls. Similarly, synthetic diet-fed VMNL rats had lower activity and concentration than chow-fed VMNL rats, but this was significant only in the case of the concentration. Synthetic diet-fed controls and chow-fed controls had comparable enterokinase concentration (Table 6).

Total lactase activity was reduced in synthetic diet-fed VMNL rats compared with their sham-lesioned controls but lactase concentration was normal. On the other hand, lactase concentration was greater in synthetic diet-fed VMNL rats than in chow-fed VMNL rats; total enzyme activity was similar between these two groups. In the case of the sham-lesioned controls, those fed the synthetic diet showed higher activity than those fed lab chow. Lactase concentration was similar in synthetic diet-fed and in chow-fed controls (Table 6).

TABLE 6
INTESTINAL PARAMETERS

Group	1	2	3	4							
N	4	4	4	6							
Operation	VMNL**	CON	VMNL	CON							
Diet	Synth	Synth	Chow	Chow	Diet*	Effect of Lesion*	Interaction*	Tukey's Test $p<$ 1–2	1–3	3–4	2–4
Mucosal Weight (mg)	462.5§ ±32.1	597.5 ±107.8	557.5 ±81.9	515.0 ±37.8	— N.S.	— N.S.	— N.S.	N.S.	N.S.	N.S.	N.S.
Mucosal Protein (mg/15 cm)	41.10 ±3.79	65.0 ±11.46	55.8 ±10.48	47.67 ±3.98	— N.S.	— N.S.	5.78† 0.05††	0.05	N.S.	N.S.	N.S.
Mucosal DNA (mg/15 cm)	2.51 ±0.28	3.60 ±0.67	3.08 ±0.64	2.67 ±0.34	— N.S.	— N.S.	— N.S.	N.S.	N.S.	N.S.	N.S.
Total Enz. Act. Entk. (U/Segment)	35.63 ±12.24	194.95 ±63.85	130.03 ±41.07	131.68 ±20.74	— N.S.	6.18 0.05	5.92 0.05	0.01	N.S.	N.S.	N.S.
Enz. Conc.-Enterokin. (U/mg Prot.)	843.8 ±231.5	2832.3 ±460.1	2242.8 ±344.0	2715.8 ±398.8	— N.S.	12.41 0.01	4.71 0.05	0.01	0.05	N.S.	N.S.
Tot. Enz. Act., Lactase (U/Segment)	0.810 ±0.059	1.276 ±0.302	0.543 ±0.080	0.778 ±0.109	7.40 0.05	6.22 0.05	— N.S.	0.05	N.S.	N.S.	0.05
Enz. Conc.-Lactase (U/mg Prot.)	19.93 ±1.89	19.95 ±2.15	10.45 ±2.31	17.13 ±3.32	4.92 0.05	— N.S.	— N.S.	N.S.	0.05	N.S.	N.S.
Tot. Enz. Act., Sucrase (U/Segment)	5.30 ±0.98	8.34 ±2.09	6.51 ±1.00	5.16 ±0.39	— N.S.	— N.S.	4.86 0.05	N.S.	N.S.	N.S.	N.S.
Enz. Con.-Sucrase (U/mg Prot.)	126.9 ±10.9	124.0 ±11.1	119.9 ±15.2	109.9 ±8.2	— N.S.	— N.S.	— N.S.	N.S.	N.S.	N.S.	N.S.
Tot. Enz. Act.-Maltase (U/Segment)	16.75 ±1.52	27.30 ±9.34	16.13 ±3.20	14.28 ±0.86	— N.S.	— N.S.	— N.S.	N.S.	N.S.	N.S.	N.S.
Enz. Conc.-Maltase (U/mg Protein)	408.6 ±12.0	396.4 ±69.9	289.1 ±13.3	303.4 ±16.3	12.87 0.01	— N.S.	— N.S.	N.S.	0.05	N.S.	N.S.

For abbreviations, see Table 3.

DISCUSSION

Since pancreatic and intestinal enzyme secretion is not only influenced by "cephalic" mechanisms but also by dietary composition [11] we investigated in the present study the effects of synthetic diets in rats that had received VMN lesions shortly after weaning (31 days of age). Such diets offer the advantage of manipulation of macronutrients and thus allow an assessment of their possible role in pancreatic and intestinal enzyme dynamics and, as assessed in the present study, of enzyme contents and concentrations. Also, earlier data had suggested [36] that—at least in the mature rat—obesity and other changes following VMH destruction are due to an exaggerated response to sensory contact with food. That tasty diets have a hyperphagia-promoting effect in VMNL rats—at least in the mature animal—has been known for many years [13, 18, 47].

The pancreatic parameters of the present study in VMNL rats and controls fed lab chow (Groups 3 and 4) show the identical pattern as did VMNL rats and controls of the above-mentioned previous study [27] in which the animals were also fed lab chow. The only exception is amylase concentration, which in the present experiment was lower and in the previous study was higher in VMNL rats than in controls (Table 6). This discrepancy may be reconciled by reports that amylase values are more variable than lipase or trypsin values [32].

Data as yet reported only in abstract form [27] indicate that VMN lesions in weanling rats retard pancreatic development. Compared with sham-lesioned controls, both pancreatic weight and protein were reduced and pancreatic DNA accumulation was arrested to about 80% of control values but showed a slight increase in time from operation to five weeks thereafter. We interpreted this increase in time of both pancreas weight and protein without concomitant DNA increase as reflecting impaired cell division but unimpaired cell enlargement. The animals of the above study were fed lab chow (Table 7).

A noteworthy finding of the present study is that pancreatic weight, protein and DNA content, total activity and concentration of trypsinogen and total activity and concentration of amylase showed the opposite changes in synthetic diet-fed and chow-fed groups of rats. It is also quite striking that there was no difference between the synthetic diet-fed and chow-fed VMNL groups, but that there were significant differences between the synthetic diet-fed and chow-fed

TABLE 7

COMPARISON OF PANCREATIC PARAMETERS IN VMNL RATS FROM A PREVIOUS REPORT* WITH THOSE OF THE PRESENT DATA (VMN LESIONED VS. SHAM-OPERATED)

Parameter	Lee *et al.* (1982) Chow	Present Study Chow	Present Study Synth. Diets
Weight	↓	↓	↑
Protein	↓	↓	↑
DNA	↓	↓	↑
Total amylase activity	NS	↓	NS
Total lipase activity	NS	NS	↑
Total trypsinogen activity	NS	NS	↑
Amylase concentration	NS	↓	NS
Lipase concentration	NS	NS	↑
Trypsinogen concentration	↑	NS	↑

*Lee *et al.* (1982).
↓=Significantly smaller than; ↑=Significantly greater than sham-operated controls.
NS=no significant difference.

sham-lesioned control groups, the synthetic-fed controls showing depressed values. Possibly, VMN destruction has had a "stimulating," "releasing" or "activating" effect on these parameters that became evident only when synthetic diets were fed. We speculate that the consequences of the VMN lesions inactivated a process that exerts its effect in sham-lesioned controls. The present data, however, do not identify this mechanism

Because digestive enzymes adapt to specific substrates in the diet [8, 20, 23, 29, 31, 39], we calculated macronutrient intake in order to assess a possible relationship between it and enzyme concentrations and activities.

During Period 1, i.e., 28 days following the hypothalamic operation when all groups of rats were fed synthetic diets, VMNL rats ate more carbyhydrate, fat and protein than their respective controls. However, during Period 2, after Groups 3 and 4 had been switched over to lab chow (14 days) intake from all three macronutrients was comparable among all groups with one exception: synthetic diet-fed controls ingested less carbohydrate than chow-fed controls. The identical macronutrient intake had been previously noted in weanling VMNL rats under the same dietary conditions [6]. At any rate, it should be noted that during Period 2 the synthetic diet-fed rats with VMNL and their controls had become normophagic, i.e., they ingested as much from all three macronutrients as the VMNL and control rats that had been switched over to lab chow.

It appears that the changes in enzyme activities and concentrations are not related to macronutrient intake, as their profiles do not show similarities. It is also highly unlikely that enzyme parameters are related to, or associated with, body weight, since the latter is comparable among all groups. Lastly, enzyme parameters do not bear any relationship to the degree or absence of obesity (Lee Index). The similar patterns of pancreas weight, protein, DNA and enzyme activities and concentrations suggest, on the other hand, an underlying common alteration in the rats with VMN lesions and an interaction with some parameters of the diet.

In contrast to the pancreatic data, small intestine parameters reveal an entirely different profile, inasmuch as mucosal weight, protein and DNA as well as sucrose content and concentration were comparable among all groups and a lesion effect was only noticeable in enterokinase activity and concentration and total lactase activity. The finding of similar maltase and sucrase activity and sucrase and lactase concentration despite differences in carbohydrate intake of the two sham-lesioned groups is contrary to Bustamente *et al.* [11]; we cannot explain this discrepancy at the present time.

Tissue levels of intestinal enzymes relevant to nutrient intake and their release into the intestinal lumen, as well as transport processes for nutrients have been suggested to be under peptidergic control [9]. Although studies in diabetic humans and animals with experimentally-induced diabetes show an involvement of the endocrine pancreas in the regulation of intestinal enzymes [32,46] recent data from our laboratories [28] indicate that exogenous insulin in otherwise intact rats has no effect on, for instance, amylase activity.

In view of the fact that VMN lesions are followed by hyperinsulinemia (e.g., [5,22]) and unquestionably affect also a number of the above secretions, it is surprising that many enzymes in the small intestine in the present study were normal. Novis *et al.* [32] have noted that, whereas vagal influence predominates the control of salivary secretion and accounts for a large part of gastric secretion, it has only minor effects on pancreatic secretion. This may not be entirely true for the weanling VMNL rat, however, as shown by alterations in at least some of the above pancreatic parameters. According to the Novis concept, one might expect a very minor effect of the vagus, via VMN lesions, on small intestinal parameters, which is indeed the case in the present study.

Since VMN lesions not only disrupt neuroendocrine events such as growth hormone and corticosterone secretion [1,5] but also neuroautonomic-metabolic functions [5,44], some of the alterations in the pancreatic parameters could be due to disruption of either of these two systems. Reduced pancreatic weight, protein and DNA levels may be due to GH deficiency in the weanling rat that is followed by microsplanchia [2]. On the other hand, some of the enzyme alterations may be due to disturbance of the hypothalamic-autonomic tone, analogous to the concept of hepatic enzyme regulation by hypothalamic structures [44].

In summary, intact rats fed synthetic diets develop atrophic levels of pancreatic weight, protein, DNA and

enzyme activities and concentrations in comparison with intact rats fed lab chow. Possibly, dietary constituents present in lab chow elicit normal pancreatic activity whereas such pancreatic stimulating agents are absent in synthetic diets; such dietary constituents may well be fiber. The above relationships apparently do not obtain for the intestine.

Experimental destruction of the ventromedial hypothalamus prevents the gross reduction of pancreatic weight, DNA, protein and enzyme parameters, i.e., it results in a dramatic disinhibition of exocrine pancreatic function. A similar disinhibition of endocrine pancreatic function is seen in both weanling and mature rats with ventromedial lesions, i.e., dramatic hyperinsulinemia.

Whereas the role of the VMN and its autonomic outflow is well established for endocrine pancreatic function, we have only scanty evidence for such an influence on the exocrine pancreas: so far the only documented phenomenon demonstrating a role of the autonomic nervous system in gastrointestinal hormone secretion is the increase of gastric secretion following vagal stimulation [21].

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