PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **185**, 49–54 (1987)

Tissue Somatostatin Levels in Three Models of Genetic Obesity in Rats (42515)

N. R. VOYLES, S. J. BHATHENA,* B. KENNEDY,* S. D. WILKINS, O. E. MICHAELIS IV,* C. M. ZALENSKI, K. I. TIMMERS, AND L. RECANT

*Diabetes Research Laboratory, Veterans Administration Medical Center, 50 Irving Street, N.W., Washington, D.C. 20422, and *Carbohydrate Nutrition Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA, Beltsville, Maryland 20705*

Abstract. A potential role for somatostatin (SRIF) in the pathogenesis of the hyperinsulinemia of obese rats was considered. SRIF like immunoreactivity (ng/mg protein) was therefore measured in hot 2 *N* acetic acid extracts of pancreas, stomach, pituitary, and hypothalamus in tissues obtained from three models of genetic obesity in rats. These models included the obese and lean controls of LA/N-*cp*, SHR/N-*cp*, and Zucker rats. To assess the effects of diet on SRIF levels, mixed diets were provided *ad lib* which contained a carbohydrate as either sucrose or starch. Some groups were fed chow diets. No significant dietary effects on tissue levels of SRIF were obtained. However, two of the three models (Zucker and SHR/N-*cp*) showed phenotypic effects on SRIF levels in pancreas; namely, obese rats showed a significantly greater concentration of SRIF ($P < 0.0005$ and < 0.0002, respectively) than did the lean littermates. These findings were confirmed by measurement of total pancreas SRIF content. Gastric levels were significantly altered only in the obese Zucker rats ($P < 0.005$) where obese tissues had lower concentrations than those of lean animals. However similar directional changes in pancreas and stomach were observed in all models. It is concluded that the hyperinsulinemia of the obese animals studied is not due to absolute deficiency in pancreatic SRIF content. It is postulated however that decreased pancreatic SRIF secretion (paracrine or otherwise) relative to pancreatic insulin content could still play a role.

The precise mechanisms for the development of hyperinsulinemia in genetically obese animals are not known. One possibility that we have considered is that alterations in pancreatic and/or gastrointestinal content or in the concentration of somatostatin (SRIF) contribute to the hyperinsulinemia. Since SRIF is a potent inhibitor of pancreatic insulin release (1) it was postulated that lower than normal levels of pancreatic SRIF might result in increased secretion of insulin into the portal and peripheral blood. Further, a lower than normal gut content of SRIF might enhance nutrient absorption and thereby also contribute to high plasma levels of insulin (2). Such a hypothesis is based on the observation that glucose absorption is impaired by SRIF (3). In the obese mouse (*ob/ob*) such a low concentration of tissue SRIF has in fact been demonstrated in both stomach and pancreas by two separate investigators (4, 5) though these findings are not supported by the work of Dolais-Kitabgi *et al.* (6). In the obese Zucker rat, we and others (7, 8) reported that although gastric levels were reduced, pancreatic levels of somatostatin were significantly higher than those in lean littermates (8).

It was therefore of interest to determine whether other genetically obese animal models show alterations in tissue SRIF levels. In this report, two newly described obese rat models were studied and compared with Zucker rats. One was the LA/N-*cp* (9), an obese, hyperinsulinemic, hyperlipemic animal, and the other was the spontaneously hypertensive rat (SHR) into which the corpulent gene was introduced to produce a nonhypertensive obese diabetic rat (SHR/N-*cp*) (10, 11). The lean littermate is hypertensive but not diabetic. During the course of these studies, the effect of dietary sucrose on tissue SRIF levels was measured since this form of carbohydrate when compared with starch has been reported to increase plasma insulin levels particularly in obese models (10).

Materials and Methods. *A. Animal models.* The animal models of obesity utilized included three genetic varieties: a new strain of LA/N-*cp* obese male rats (*cp/cp*) and their lean littermates (+/?) (9), female Zucker rats (*fa/fa*) and their lean littermates (+/?) (7, 8), and the most recently described model, the obese male SHR/N-*cp* (*cp/cp*) and lean littermates (+/?) (10, 11). Zucker rats were purchased from Dr.

0037-9727/87 $1.50

Julia Clarke of the Department of Pharmacology at the University of Indiana (Indianapolis, Indiana). The other animals were obtained from Dr. O. E. Michaelis (Carbohydrate Nutrition Laboratories, USDA, Beltsville MD,) and Dr. Carl Hansen (Small Animal Section, Veterinary Resources Branch, NIH, Bethesda, MD).

All animals were approximately 4 weeks of age at the onset of dietary treatment. They were maintained at constant temperature on a 12-hr reversed light/dark cycle with unlimited access to food. They were fed diets containing 54% carbohydrate as either sucrose or cornstarch plus 10% casein, 10% lactalbumin, 5.9% cellulose, 4% beef tallow, 4% lard, 4% corn oil, 4% hydrogenated coconut oil 3% AIN salt mix, and 1% vitamin fortification mix (9) for a periods of 6–12 weeks. Additional groups of Zucker and SHR rats received standard chow diets for the same time periods. Sacrifice was carried out at a fixed time (11:00 AM to 1:00 PM) after overnight fasting except as indicated in Table I. Animals were brought into the room individually and decapitated. Trunk blood was collected in tubes containing 1000 U Trasylol (FBA Pharmacia, New York, NY) and 10.5 mg EDTA. Plasma was separated and stored at −70°C; glucose was measured by an enzymatic method (hexokinase) utilizing the centrifichem analyzer. Plasma insulin was measured by the double-antibody method of Morgan and Lazarow (12). Pancreatic rat insulin was a gift from Eli Lilly and utilized as a standard in the insulin assays.

B. Tissue extracts. Pancreas, stomach, pituitary, and hypothalamus were rapidly dissected, rinsed in cold saline, and frozen on dry

TABLE I. WEIGHT GAIN, PLASMA GLUCOSE AND INSULIN IN THREE MODELS OF GENETICALLY OBESE AND LEAN RATS FED DIFFERENT DIETS

Strain	Phenotype	Diet	No. of rats	Weight gain[b] (g)	Plasma glucose (mg/100 ml)	Plasma unsulin (μU/ml)
LA/N-cp	Lean	Starch	6	252 ± 7 (a)[c]	81 ± 11 (a)	65 ± 8 (a)
	Lean	Sucrose	6	282 ± 12 (a)	97 ± 7 (a, b)	75 ± 14 (a)
	Obese	Starch	6	501 ± 21 (b)	63 ± 9 (a)	433 ± 84 (b)
	Obese	Sucrose	6	518 ± 16 (b)	111 ± 3 (b)	496 ± 71 (b)
SHR/N-cp	Lean	Starch	4	361 ± 19 (a)	93 ± 6 (a)	228 ± 102 (a)
	Lean	Sucrose	5	405 ± 6 (a)	91 ± 2 (a)	178 ± 45 (a)
	Obese	Starch	4	576 ± 13 (b)	92 ± 3 (a)	3377 ± 531 (b)
	Obese	Sucrose	5	563 ± 16 (b)	135 ± 21 (a)	3385 ± 202 (b)
SHR/N-cp[a]	Lean	Chow	18	301 ± 30 (a)	123 ± 4 (a)	276 ± 47 (a)
	Obese	Chow	18	427 ± 21 (b)	325 ± 13 (b)	4053 ± 547 (b)
Zucker	Lean	Starch	6	184 ± 10 (a)	106 ± 2 (a, b)	39 ± 6 (a)
	Lean	Sucrose	6	172 ± 13 (a)	108 ± 5 (a, b, c)	66 ± 12 (a)
	Lean	Chow	6	135 ± 4 (b)	98 ± 8 (a)	50 ± 5 (a)
	Obese	Starch	6	350 ± 11 (c)	113 ± 2 (a, b, c)	144 ± 20 (b)
	Obese	Sucrose	6	322 ± 7 (d)	122 ± 5 (c)	196 ± 22 (b)
	Obese	Chow	6	284 ± 8 (e)	115 ± 2 (b, c)	150 ± 30 (b)
					Anova	
LA/N-cp		Phenotype		$P < 0.0001$	NS	$P < 0.0001$
		Diet		NS	$P < 0.05$	NS
SHR/N-cp (fasted)		Phenotype		$P < 0.0003$	NS	$P < 0.0001$
		Diet		NS	NS	NS
SHR/N-cp (fed)		Phenotype		$P < 0.0003$	$P < 0.0001$	$P < 0.0001$
Zucker		Phenotype		$P < 0.0001$	$P < 0.005$	$P < 0.001$
		Diet		$P < 0.0001$	NS	NS

[a] Rats were sacrificed in fed state.
[b] Different letters (a–e) indicate significant differences in mean values within a group.
[c] Mean ± SEM.

ice. The tissues were then extracted for 10 min in boiling 2 *N* acetic acid, chilled on ice, and homogenized with a Brinkman polytron at setting No. 5 for 20–30 sec. An aliquot was removed for protein determination (13), and the remainder was centrifuged at 4°C for 10 min at 12,000*g*. The supernatant was quickly separated, lyophilized, and stored at −20°C until assayed for SRIF. SRIF was assayed by radioimmunoassay as previously described and validated (14). SRIF antibody was a gift from Dr. John E. Gerich.

Statistical analysis was carried out by randomized complete block analysis of variance (ANOVA) to test the influence of phenotype and diet upon tissue levels of SRIF.

Results. The diets, the number of animals in each group, the body weight gain, and the plasma insulin (IRI) and glucose levels are shown in Table I. It may be seen that significant increments in weight and elevated levels of plasma insulin are present in the obese rats of all three genetic models. These effects are phenotypically determined. A phenotypic effect on plasma glucose was seen in the Zucker rats and in the SHR rats fed a chow diet. The obese animals manifested higher glucose levels than did the lean controls. With regard to dietary effects, the LA/N rats, both lean and obese, showed significantly higher glucose levels on sucrose diets than on starch diets. Plasma glucose levels were lower in overnight-fasted SHR animals (lean and obese) than in those which had not been fasted prior to sacrifice. Table II shows the content of SRIF in pituitary, hypothalamus, stomach, and pancreas of the three rat models expressed per milligram tissue protein. The only significant effects were phenotypically determined. The gastric concentration of SRIF was lower in the obese Zucker rats than in their lean controls as we previously reported (8). Although the levels also tended to be lower in the other obese models, the differences were not statistically significant. Starch-fed obese SHR rats showed lower hypothalamic concentrations of SRIF while obese LA/N animals showed mild increases in pituitary levels.

The major findings occurred in the pancreatic concentrations of SRIF. In the Zucker rats and the obese SHR animals, SRIF concentrations were significantly elevated compared with the lean controls. The obese LA/N-*cp* rats fed sucrose also showed higher concentrations of SRIF though the differences from controls were not statistically significant. Dietary composition appeared to have no influence on SRIF concentrations in any of the tissues of the animals studied.

In order to better assess the meaning of the altered SRIF concentrations in pancreas, total organ content of SRIF was measured and plotted in Fig. 1. In confirmation of the concentration data, total pancreatic SRIF levels in the obese Zucker and SHR-*cp* animals were significantly higher than those in the respective lean controls. The levels in the obese LA/N-*cp* animals were slightly higher but still not significantly different from their controls.

Discussion. Since the tissue content of a peptide is dependent upon many factors, such as rates of biosynthesis, degradation, and secretion, it should be recognized that the presence of decreased or increased levels in a tissue does not automatically imply decreased or increased secretion of the pepide. Further, evaluation of the effects of neuroendocrine peptides, such as SRIF, also require consideration of the likelihood of paracrine effects since in pancreas SRIF is found in D cells of the islets of Langerhans in close proximity to B or insulin-secreting cells. With these considerations in mind, we have examined the tissue concentrations and content of SRIF in three obese rat models. It is clear that the hyperinsulinemia in the obese animals is not associated with levels of pancreatic SRIF lower than those found in their lean littermates. In fact, pancreatic SRIF content was significantly higher in the obese Zucker and SHR rats and higher (though not significant) in the LA/N obese rats when compared with their respective controls.

In a similar vein, gastric levels did not appear to be involved in the hyperinsulinemia since, although lower in the obese Zucker rats than in their controls, the differences in the other obese models were not significant. It is of interest however that the directions of SRIF change in the rat models were similar to each other.

The highest pancreatic levels of SRIF were obtained in the obese diabetic model (SHR rats). This is not surprising since in many instances (15, 16), though not all (17), diabetes is associated with an increased content of SRIF in the pancreas. The rat models, however, contrast against the obese hyperinsulinemic mouse models (C57Bl/6J *ob/ob*) in that pan-

TABLE II. EFFECT OF DIET AND PHENOTYPE ON TISSUE SOMATOSTATIN CONTENT IN THREE MODELS OF GENETICALLY OBESE AND LEAN RATS

Strain	Phenotype	Diet	No. of rats	Pituitary[b]	Hypothalamus	Stomach	Pancreas
				(ng/mg protein)			
LA/N-cp	Lean	Starch	6	1.01 ± 0.14 (a)[c]	88.85 ± 24.3 (a)	3.32 ± 0.73 (a)	1.55 ± 0.27 (a)
	Lean	Sucrose	6	0.93 ± 0.15 (a)	46.37 ± 11.2 (a)	2.82 ± 0.75 (a)	1.55 ± 0.29 (a)
	Obese	Starch	6	1.75 ± 0.35 (b)	51.69 ± 7.3 (a)	2.92 ± 0.66 (a)	1.57 ± 0.36 (a)
	Obese	Sucrose	6	1.32 ± 0.12 (a, b)	52.55 ± 4.8 (a)	2.75 ± 0.62 (a)	2.26 ± 0.52 (a)
SHR/N-cp	Lean	Starch	4	1.76 ± 0.83 (a, b)	91.43 ± 10.63 (a)	—	1.22 ± 0.27 (a)
	Lean	Sucrose	5	0.74 ± 0.30 (a)	87.43 ± 8.60 (a)	—	0.63 ± 0.11 (a)
	Obese	Starch	4	1.58 ± 0.58 (a, b)	44.78 ± 4.95 (b)	—	3.34 ± 0.63 (b)
	Obese	Sucrose	5	2.96 ± 0.64 (b)	84.28 ± 14.72 (a)	—	3.31 ± 0.52 (b)
SHR/N-cp[a]	Lean	Chow	18	2.20 ± 0.43 (a)	278.2 ± 41.7 (a)	2.01 ± 0.11 (a)	1.68 ± 0.19 (a)
	Obese	Chow	18	2.13 ± 0.31 (a)	208.1 ± 31.8 (a)	1.71 ± 0.12 (a)	2.91 ± 0.36 (b)
Zucker	Lean	Starch	6	1.20 ± 0.12 (a)	70.71 ± 9.5 (a)	1.16 ± 0.20 (a, b)	0.76 ± 0.10 (a)
	Lean	Sucrose	6	1.18 ± 0.30 (a)	121.26 ± 29.5 (a)	0.76 ± 0.09 (b, c)	0.93 ± 0.14 (a)
	Lean	Chow	6	1.34 ± 0.30 (a)	92.02 ± 15.4 (a)	1.28 ± 0.20 (a)	1.44 ± 0.16 (a, b)
	Obese	Starch	6	1.22 ± 0.18 (a)	114.32 ± 34.8 (a)	0.87 ± 0.06 (a, b, c)	1.47 ± 0.25 (a, b)
	Obese	Sucrose	6	0.80 ± 0.07 (a)	73.14 ± 12.2 (a)	0.71 ± 0.10 (c)	2.30 ± 0.46 (c)
	Obese	Chow	6	0.93 ± 0.11 (a)	78.40 ± 13.0 (a)	0.68 ± 0.09 (c)	1.73 ± 0.21 (b, c)
				Anova			
LA/N-cp		Phenotype		$P < 0.02$	NS	NS	NS
		Diet		NS	NS	NS	NS
SHR/N-cp (fasted)		Phenotype		NS	$P < 0.005$	—	$P < 0.0002$
		Diet		NS	NS	—	NS
SHR/N-cp (fed)		Phenotype		NS	NS	NS	$P < 0.005$
Zucker		Phenotype		NS	NS	$P < 0.005$	$P < 0.0005$
		Diet		NS	NS	NS	NS

[a] Rats were sacrificed in fed state.
[b] Different letters (a–c) indicate significant differences in mean values within a group.
[c] Mean ± SEM.

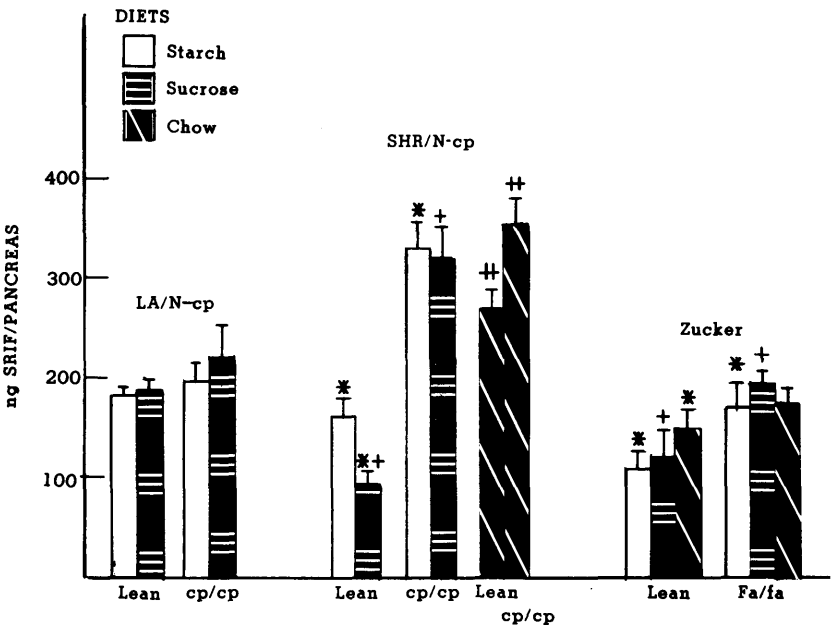

FIG. 1. Total pancreatic SRIF (ng) for three strains of corpulent rats and lean littermates studied on three different diets. All animals were killed after an overnight fast except the chow-fed SHR/N-*cp* animals. Within each strain, bars with the same symbol were significantly different ($P < 0.05$).

creatic SRIF concentrations are lower in the obese mice (4, 5). In the C57Bl/KsJ (*db/db*) obese–diabetic mice, however, pancreatic SRIF was unchanged during the early phase of the obese–diabetic syndrome (5, 18), while in the later stages the SRIF levels rose and plasma insulin levels decreased. It would appear that the failure to see decreased pancreatic SRIF in the obese *db/db* mice was associated with the diabetic syndrome as compared with the less complicated *ob/ob* nondiabetic syndrome.

As noted priorly, the SRIF content of a tissue may or may not reflect the secretory pattern. Unfortunately, measurements of SRIF secretion from perfused rat pancreas have not met with success in our laboratory or that of Gerich (personal communication). It is perhaps secretion which may be critical in terms of the metabolic effects of the peptide. In streptozotocin or alloxan diabetic rats it is apparent that both increased pancreatic SRIF content and secretion have been described (15, 19). In two separate studies of isolated pancreas perfusions in obese rats (Zucker and Wistar fatty rat) SRIF secretion following arginine was also increased compared with lean controls (20, 21). The pancreatic content of SRIF was increased in the Zucker (7, 8) and not measured in the other obese animals.

It may be that the ability of pancreatic SRIF to regulate insulin secretion is dependent upon the ratio of B cells (insulin secreting) to D cells (SRIF secreting). In the obese Zucker rat, Seino *et al.* (20) showed that the insulin response to arginine was increased 10-fold compared to lean controls while the SRIF response was increased only 3-fold. In the streptozotocin diabetic, the SRIF response was quantitatively similar to that in the obese rat (19) though obviously the available B cells and hence insulin responses were markedly reduced. These findings suggest that either the B cells in obese rats are resistant to inhibition by SRIF or, considering the larger number of functional B cells, more SRIF is necessary to inhibit their secretion.

Two other features of our observations deserve comment. Although the presence of SRIF in the anterior pituitary has not previously been described, our findings indicate that whole-rat pituitary contains significant amounts of SRIF-like immunoreactive material. At this point in time we do not know whether the SRIF represents uptake and internalization or contamination from hypo-

thalamic-portal blood or whether all the measured SRIF is contributed by the posterior pituitary and stalk as reported by Patel *et al.* (22).

Finally in the present studies, there were no significant effects of dietary sucrose, as compared with starch, on tissue SRIF in any of the obese models and their lean controls. However, neither was there any diet effect on fasting plasma insulin values. An effect of sucrose-containing diets on fasting plasma insulin was not invariably found (10, 11, 23).

We thank Ms. C. Ortiz for typing this manuscript. The work was supported by funds from the Washington, D.C. Area Affiliate of the American Diabetes Association, the Veterans Administration, and the U.S. Department of Agriculture, Beltsville, Maryland.

1. Curry DL, Bennett LL, Li CH. Direct inhibition of insulin secretion by synthetic somatostatin. Biochem Biophys Res Commun **38**:885–887, 1974.
2. Schusdziarra V, Harris V, Arimura A, *et al.* Evidence for a role of splanchnic somatostatin in the homeostasis of ingested nutrients. Endocrinology **104**:170–178, 1978.
3. Reichlin S. Medical Progress, Somatostatin (Second of Two Parts). N Engl J Med **309**:1556–1563, 1983.
4. Patel CY, Orci L, Bankier A, *et al.* Decreased pancreatic somatostatin (SRIF) concentration in spontaneously diabetic mice. Endocrinology **99**:1415–1418, 1976.
5. Berelowitz M, Coleman DL, Frohman LA. Temporal relationship of tissue somatostatin-like immunoreactivity to metabolic changes in genetically obese and diabetic mice. Diabetes **29**:717–723, 1980.
6. Dolais-Kitabgi J, LeMarchand-Brustel Y, Freychet P. Somatostatin in the pancreas and hypothalamus of obese mice. Diabetologia **17**:257–261, 1979.
7. Sheppard MC, Shapiro B, Hudson A, Pimstone BL. Tissue and serum somatostatin-like immunoreactivity in lean and obese Zucker rats. Horm Metab Res **12**: 236–239, 1980.
8. Voyles NR, Awoke SS, Wade A, Bhathena SJ, Smith SS, Recant L. Starvation increases gastrointestinal somatostatin in normal and obese Zucker rats. Horm Metab Res **14**:392–395, 1982.
9. Bhathena SJ, Revett R, Michaelis OE, Ellwood KC, Voyles NR, Recant L. Genetic obesity and dietary sucrose decrease hepatic glucagon and insulin receptors in LA/N-cp rats. Proc Soc Exp Biol Med **181**:71–77, 1986.
10. Michaelis OE, Ellwood KC, Judge JM, Schoene NW, Hansen CT. Effect of dietary sucrose on the SHR/N-cp rat. Amer J Clin Nutr **39**:612–618, 1984.
11. Michaelis OE, Patrick DH, Hansen CT, Canary JJ, Werner RM, Carswell N. Insulin-independent diabetes mellitus (Type II). Spontaneous hypertensive/NIH corpulent rat. Amer J Physiol **123**:398–400, 1986.
12. Morgan CR, Lazarow A. Immunoassay of insulin: Two antibody system. Diabetes **12**:115–126, 1963.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biochem **193**:265, 1951.
14. Recant L, Voyles NR, Wilkins SD, Fairtile RD, King C. Role of the hypophysis in regulation of pancreatic and gastric somatostatin. Endocrinology **115**:2260–2265, 1984.
15. Patel Y, Cameron D, Bankies A, *et al.* Changes in somatostatin concentration in pancreas and other tissues of streptozotocin diabetic rats. Endocrinology **103**:917–924, 1978.
16. Gerich JE. Somatostatin and diabetes. Amer J Med **70**:619–626, 1981.
17. Peterson B, Elde R, Efendic S, *et al.* Somatostatin in the pancreas, stomach and hypothalamus of the diabetic chinese hamster. Diabetologia **13**:463–466, 1977.
18. Timmers K, Voyles NR, Zalenski C, Wilkins S, Recant L. Altered β-endorphin, met- and leu-enkephalins and enkaphalin-containing peptides in pancreas and pituitary of genetically obese–diabetic (*db/db*) mice during development of the diabetic syndrome. Diabetes, 1986, in press.
19. Kadowaki S, Taminato T, Chiba T, *et al.* Somatostatin release from the isolated, perfused diabetic rat pancreas. Inverse relationship between pancreatic somatostatin and insulin. Diabetes **29**:960–963, 1980.
20. Seino S, Seino Y, Takemura J, *et al.* Somatostatin, insulin and glucagon secretion in obese rats. Amer J Physiol **241**:E146–150, 1981.
21. Seino Y, Seino S, Usami M, *et al.* Somatostatin, glucagon, and insulin secretion from isolated perfused pancreas of new genetically obese hyperglycemic rats. Metabolism **33**:429–431, 1984.
22. Patel YC, Zingg HH, Dreifuss JJ. Calcium-dependent somatostatin secretion from rat neurohypophysis in vitro. Nature (London) **267**:852–853, 1977.
23. Michaelis OE IV, Ellwood KC, Emberland JJ, Hansen CT, Canary JJ. Effect of dietary carbohydrate on expression of diabetes in the SHR/N-corpulent rat: A new model for obesity induced diabetes. J Obesity Weight Reg. **4**:168–178, 1985.

Received July 21, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted January 15, 1987.