Monoamine Oxidase, Catechol-O-methyltransferase, and Norepinephrine Levels in Mice with the Hereditary Obese-hyperglycemic Syndrome

Jerome M. Feldman, M.D., and Joan H. Henderson, M.D., Durham

SUMMARY

There is some evidence that there are abnormalities in catecholamine metabolism in patients with obesity and diabetes mellitus. In this study we determined the tissue levels of catecholamine-metabolizing enzymes (monoamine oxidase [MAO] and catechol-O-methyltransferase [COMT]) and norepinephrine (NE) in mice with the hereditary obese-hyperglycemic syndrome (ob/ob mice). The ob/ob mice have greater MAO activity in their kidneys (+ 43 per cent), testes (+ 51 per cent), and white adipose tissue (+ 87 per cent) than their normal-weight littermates. There is no difference in MAO activity of pancreas, liver, brain, brown fat, heart, and islets of Langerhans in ob/ob and normal mice. The ob/ob mice have less COMT activity in their liver (− 39 per cent) than normal mice. There is no difference in COMT activity in the kidney, testis, pancreas, and brain between ob/ob and normal mice. The Michaelis constant of kidney MAO for the tryptamine substrate is similar in normal and obese mice, suggesting that the increased MAO activity is due to a greater amount of tissue MAO rather than an altered affinity of MAO for the tryptamine substrate.

The ob/ob mice have a persistent elevation in MAO activity despite euglycemia (older mice) and a reduction in weight (caloric restriction), suggesting that the elevated MAO activity in ob/ob mice is a primary rather than a secondary disturbance. The total NE content of kidney, white fat, and brown fat is similar in ob/ob and normal mice. The islet NE content is less than 3 pg. per islet in ob/ob and normal mice. DIABETES *27*:389-95, April, 1978.

Recent observations suggest that some obese patients and some patients with diabetes mellitus may have an increase in catecholamine production. Obese women excrete greater amounts of 3-methoxy-4-hydroxymandelic acid (vanilmandelic acid, or VMA) in their urine than normal-weight women of comparable age.[1] This increase is noted even when the VMA excretion is corrected for the urinary excretion of creatinine. Halter and Porter noted that plasma catecholamine concentration was elevated in one third of a group of patients with nonketotic diabetes.[2] In contrast, Cryer et al. noted that the mean plasma norepinephrine (NE) and epinephrine (E) concentration of a group of 51 nonketotic diabetic patients did not differ from that of a group of 35 nondiabetic control subjects.[3] Christensen reported clearly elevated plasma catecholamine levels in ketoacidotic diabetics as well as an exaggerated catecholamine response to exercise.[4] He found normal plasma catechomamine levels in nonketotic diabetic subjects.

Catecholamines are inactivated by the enzymes monoamine oxidase (monoamine:oxygen oxidoreductase, 1.4.3.4, MAO) and catechol-O-methyltransferase (S-adenosylmethionine: catechol-O-methyltransferase, 2.1.1.6, COMT).[5] It is not known if the activity of either of these important enzymes is altered in the tissues of obese or diabetic subjects.

The ob/ob-genotype mouse, described by Ingalls et al. in 1950, inherits obesity and diabetes as Mendelian recessive traits.[6] By the age of four to five months the ob/ob mice are twice as heavy as their heterozy-

From the Durham Veterans Administration Hospital and the Division of Endocrinology, Department of Medicine, Duke University Medical Center, Durham, North Carolina.

Accepted for publication October 10, 1977.

gous (ob/+) or homozygous (+/+) littermates. They also have heavier organs (liver, adrenal glands) than their normal-weight littermates. There are alterations in the enzymatic activity of some of their endocrine and nonendocrine organs.[7,8]

Although the adrenal glands of the ob/ob mice are more than twice as large as those of their normal-weight littermates, the enlargement is due to the cortical rather than the medullary part of the organ.[9] Histochemical techniques suggest that the adrenal medulla has a normal catecholamine content.[9] There is no information available concerning the plasma catecholamine concentration, the urinary catecholamine excretion, or the tissue activity of catecholamine-metabolizing enzymes in these obese diabetic mice. In the present study we compare the tissue MAO activity, COMT activity, and NE concentration of obese-diabetic mice and their normal-weight littermates.

MATERIAL AND METHODS

Experimental animals. The mice were delivered to our medical center from the Jackson Memorial Laboratory (Bar Harbor, Me.) when they were one month old. The animals used in this study were male obese mice, strain C57BL/6J, genotype ob/ob (hereafter referred to as obese mice) and their normal-weight male littermates. The normal-weight mice (hereafter referred to as normal mice) were either heterozygous (genotype ob/+) or homozygous (genotype +/+). The per cent composition by weight of the Purina Lab Chow (Ralston Purina Co., St. Louis) that the mice ate was 23 per cent protein, 51 per cent carbohydrate, and 4.5 per cent fat. The obese and normal littermates were kept in the same cage. The mice were kept in a room with outside windows. The fluorescent lights in the room were turned on from 0800 to 1630 hours.

Two series of experiments were performed. At the time of the first series, the mice were two to 4½ months old. An obese mouse and his littermate were studied on the same day or on sequential days in random order. Food was removed from the cages 12 hours before the experiment. The mice were killed by cervical dislocation, and blood was obtained for plasma glucose. At the time of killing the normal mice weighed 26.3 ± 0.60 gm. (M. ± S.E.M.) and the obese mice weighed 51.4 ± 1.76 gm. The plasma glucose of the normal mice ranged from 86 to 154 mg./dl. (mean 110 mg./dl.), and the plasma glucose of the obese mice ranged from 172 to 256 mg./dl. (mean 221 mg./dl.).

Kidneys, testes, pancreas, liver, and brain were removed from the animals and were weighed. Weighed samples of kidney cortex, testis, pancreas, and liver as well as the entire brain were homogenized in 1.1 per cent potassium chloride. An aliquot of each tissue was analyzed for its protein content; additional aliquots were diluted with an appropriate phosphate buffer, in preparation for the enzyme assays. Representative sections were prepared from some of the normal and obese mice for microscopic examination. After fixation in 10 per cent formalin they were stained with hematoxylin and eosin and examined under a light microscope.

When mitochondrial-rich pellets were prepared, a kidney was homogenized with a glass pestle in a solution (pH 7.8) of the following composition: 0.25 M sucrose, 1.1 per cent potassium chloride, and 0.01 M potassium phosphate. The homogenate was centrifuged in a refrigerated centrifuge (4° C.) at 90×g to remove nuclei, cell membranes, and unbroken cells. The supernatant was then centrifuged at 4° C. at 27,700×g for 15 minutes. The supernatant was aspirated and discarded. The pellet was suspended in potassium chloride, and aliquots of the suspension were then analyzed for protein content and MAO activity.

In the second series of experiments, one-month-old obese mice were randomly divided into two groups. The first group remained with their normal littermates and had continuous access to food. The second group of obese mice was placed in separate cages and given access to food for two to three hours per day. The mice were weighed weekly and the duration of time that food was available to them was adjusted so that their weight was intermediate between the weight of the normal and obese mice: This group of obese diet-restricted mice is hereafter referred to as obese-DR. The obese-DR mice were more active physically than the obese mice; they ate voraciously when food was presented to them. The mice in this series of studies were older than those in the first series. Their ages ranged from 4½ to seven months. A normal, an obese, and an obese-DR were studied on each of the experimental days. At the time of killing, the weights of the mice were (M. ± S.E.M.): normal 24.9 ± 0.89 gm., obese 45.5 ± 1.12 gm., and obese-DR 32.8 ± 1.7 gm. The mean glucose concentration of the three groups of mice in milligrams glucose per deciliter plasma were: normal, range 60 to 179, with a mean of 136; obese, range 60 to 330, with a mean of 138; and obese-DR, range of 50-212, with a mean of 112. It

was noted previously that the plasma glucose concentration of obese mice may return to normal at six to seven months of age.[10]

Kidneys, testes, liver, brain epididymal white fat, interscapular brown fat, and the heart were removed from the animals and weighed. Weighed samples of kidney cortex, testis, liver, white fat, brown fat, and the entire brain and heart were homogenized in 1.1 per cent potassium chloride. An aliquot of each tissue was analyzed for its protein content; additional aliquots were diluted with an appropriate phosphate buffer and analyzed for MAO activity. Samples of kidney cortex, white fat, and brown fat were weighed and homogenized in 0.1 M perchloric acid (3° C.) in a ground-glass homogenizer. After the homogenate was centrifuged at 4,000×g, the supernatant was analyzed for NE. Pancreatic tissue was removed from the normal, obese, and obese-DR mice. Pancreatic islets were isolated by collagenase digestion in a modification of the protocol of Lacy and Kostianovsky.[11,12] Some of the isolated islets were homogenized in 1.1 per cent potassium chloride for protein and MAO activity. Other islets were homogenized in 0.1 M perchloric acid and anaylzed for their NE content.

Analytic methods. The MAO activity of tissue homogenates was determined with a sensitive radioassay that employed ^{14}C tryptamine as a substrate.[13] The MAO activity of the homogenates was equal to the amount of ^{14}C-labeled indoleacetaldehyde and indoleacetic acid formed. These metabolites were isolated by differential solvent extraction and counted in a liquid scintillation counter. With the exception of the pancreatic islets, the tissues were assayed with 1 mM tryptamine. This is a saturating concentration of substrate. Because of the small quantity of islet homogenate available, islet MAO was determined with 0.35 μM tryptamine. This concentration of tryptamine gave maximal sensitivity. In studies to determine the Michaelis constant (Km) and the maximum velocity of the enzyme reaction (Vmax), kidney homogenates and kidney mitochondrial-rich pellets were incubated with six concentrations of tryptamine ranging from 5 to 50 μM. The data were then analyzed by the double-reciprocal plot method of Lineweaver-Burke.[14] COMT activity was assayed with a radioassay method employing ^{14}C-S-adenosylmethionine (SAM) and nonradioactive 3,4-dihydroxybenzoic acid.[15] The COMT activity was equal to the ^{14}C-O-methylated benzoate derivative that was formed. After isolation by differential solvent extraction, the ^{14}C-O-methylated benzoic acid was counted in a liquid scintillation counter. The substrate concentrations used (SAM 60 μM and 3,4 dihydroxybenzoic acid 3mM) gave maximal enzyme activity. After aliquots of tissue homogenates were digested with 0.5 M NaOH (room temperature for 30 minutes), their protein content was determined by the Lowry method.[16] The specific activity (SA) of the MAO and COMT activity is expressed as pmoles of product formed per milligram of tissue protein per minute. Each determination of MAO and COMT was done in duplicate with two different-sized aliquots of tissue homogenate. The SAs in the two aliquots were in excellent agreement, indicating that the substrate concentration used was never rate-limiting. The NE method uses partially purified bovine adrenal gland phenylethanolamine-N-methyltransferase (PNMT) to transfer a ^{3}H-labeled methyl group from ^{3}H-SAM to NE.[17] After appropriate purification steps, the radioactive E that has been formed is quantitated by liquid scintillation counting. Because of the differences in the nonradioactive SAM content in different types of tissues, the NE content of each tissue is measured with its own internal standard. The assay sensitivity is 50 pg. NE per tube. The NE content is expressed as picograms NE per milligram tissue protein. Plasma glucose was determined with a glucose oxidase method.[18]

Chemicals. Radioisotopes and chemicals were purchased from the following sources: Tryptamine bisuccinate (side chain-2-^{14}C, SA 60 mCi. per μmole), S-adenosyl-L-methionine (methyl-^{14}C, SA 40-60 mCi. per mmole), S-adenosyl-L-methionine (methyl-^{3}H, SA 7.5 Ci. per mmole) (New England Nuclear, Boston); 3,4 dihydroxybenzoic acid and tryptamine HCl (Cal Biochem, La Jolla, Ca.); and S-adenosylmethionine iodide (Sigma Chemical, St. Louis).

Statistics. Standard statistical methods were used to study the data.[19] In the first series of experiments, in which two groups (normal and obese) are compared, the Student's *t*-test was used to analyze the differences between the groups. In the second series of experiments, in which obese and obese-DR mice were compared with normal mice, the data were subjected to analysis of variance. The significance of the differences between the mean value of the normal mice and the mean value of the other two groups was then determined with Dunnett's single-tailed multiple comparison test. The Pearson-product correlation coefficient (r) was used for correlation analysis. Unless otherwise

TABLE 1

MAO and COMT activity of homogenates of organs from normal and obese mice

Enzyme activity	Tissue	Specific activity (pmoles indoleacetic acid/mg. tissue protein/min.)*		Per cent change	Significance
		Normal	Obese		
MAO	Kidney	306 ± 20.8	437 ± 27.6	+ 43.0	$P < 0.01$
	Testis	219 ± 23.7	330 ± 38.6	+ 50.7	$P < 0.05$
	Pancreas	488 ± 40.3	600 ± 38.6	+ 22.9	NS.†
	Liver	1,012 ± 84.0	1,275 ± 103.8	+ 26.0	NS.
	Brain	1,606 ± 85.9	1,517 ± 57.9	− 5.5	NS.
COMT	Kidney	181 ± 18.6	188 ± 20.4	+ 3.8	NS.
	Testis	32 ± 3.4	37 ± 4.2	+ 15.6	NS.
	Pancreas	30 ± 2.0	31 ± 3.1	+ 3.3	NS.
	Liver	577 ± 51.0	416 ± 34.7	− 27.9	$P < 0.02$
	Brain	31 ± 3.6	33 ± 4.9	+ 6.4	NS.

*Each value is Mean ± S.E.M. of 11 mice.
†NS. = nonsignificant.

stated, the data are expressed as mean ± S.E.M.

RESULTS

Table 1 demonstrates that homogenates of kidney and testis from obese mice have significantly greater MAO activity than the homogenates of kidney and testis from normal mice. The MAO activity of pancreas, liver, and brain of obese mice did not differ significantly from the MAO activity of these organs in normal mice. The COMT activity in homogenates of liver from obese mice was significantly lower than the COMT activity in homogenates of liver from normal mice (table 1). The COMT activity of kidney, testis, pancreas, and brain of obese mice was not significantly different from the COMT activity of these organs in normal mice. It is most appropriate to express enzyme activity per milligram of tissue protein, as we have done. However, when the data are recalculated on the basis of enzyme activity per milligram of tissue wet weight, the obese mice also have greater MAO activity in their kidneys and testes and lower COMT activity in their livers than the normal mice.

Table 2 depicts the results of a series of experiments to compare the Michaelis constant (Km) and maximum velocity (Vmax) of kidney MAO in normal and obese mice. These studies also compared the Vmax determined from a kinetic analysis of the data obtained at six different tryptamine concentrations (5 to 50 μM) with the Vmax determined with the saturating tryptamine concentration (1 mM) used in the majority of the MAO analyses in this report. We assayed the MAO activity of kidney homogenates and the MAO activity of mitochondrial-rich pellets prepared from kidney. There was no difference in the Km of the homogenate MAO in normal and obese mice. There was no difference in the Km of pellet MAO in normal and obese mice. Both kinetic analysis and direct measurement with saturating concentrations of tryptamine indicated that the Vmax of the kidney homogenate MAO was greater in the obese mice than in the normal mice. Both forms of analysis also

TABLE 2

KM and Vmax of MAO in homogenate and pellet obtained from kidneys of four normal and four obese mice

Tissue preparation	Km (μM)*		Vmax (pmoles idoleacetic acid/mg. tissue protein/min.)			
			Kinetic analysis§		Direct measurement†	
	Normal	Obese	Normal	Obese	Normal	Obese
Homogenate‡	7 ± 0.8	10 ± 1.4	293 ± 25§	426 ± 9§	303 ± 23§	401 ± 11§
Pellet‡	11 ± 0.6	15 ± 1.8	543 ± 41§	785 ± 28§	550 ± 51§	775 ± 36§

*Obtained from double-reciprocal plot analysis of MAO activity with tryptamine concentrations ranging from 5 to 50 μM.
†MAO activity with tryptamine concentration of 1 mM.
‡Each value is Mean ± S.E.M. of kidneys from four mice.
§Vmax of kidney MAO from obese mice differs from Vmax of kidney MAO from normal mice with $P < 0.01$.

TABLE 3

MAO activity in homogenates of organs from normal, obese, and obese-DR mice

Tissue	MAO activity (pmoles indoleacetic acid/mg. tissue protein/min.)* Normal	Obese	Obese-DR
Kidney	323 ± 20	489 ± 34†	500 ± 26†
Testis	240 ± 19	355 ± 28‡	460 ± 58†
Liver	1,123 ± 95	1,127 ± 84	1,342 ± 92
Brain	1,492 ± 63	1,479 ± 88	1,459 ± 91
White fat	561 ± 49	1,048 ± 144†	1,359 ± 199†
Brown fat	369 ± 42	619 ± 103	652 ± 75
Heart	198 ± 10	207 ± 24	227 ± 11
Pancreatic islets	8.3 ± 1.1	10.2 ± 1.7	8.7 ± 1.9

*Each value is Mean ± S.E.M. of eight mice. The tryptamine concentration used to measure islet MAO was 0.35 μM. The tryptamine concentration used to measure all other tissues was 3 mM.

†Differs significantly from normal with $P < 0.01$.

‡Differs significantly from normal with $P < 0.05$.

showed that the Vmax of the mitochondrial-rich pellet was greater in the obese mouse than in the normal one. Finally, there was good agreement between the Vmax values obtained by kinetic analysis and the Vmax values obtained by direct measurement. This agreement was present in the homogenate MAO from normal mice, the homogenate MAO from obese mice, the pellet MAO from normal mice, and the pellet MAO from obese mice. As MAO is predominantly located in mitochondria, the specific activity (SA) of the MAO in the pellets was greater than the SA of the MAO in the homogenates.

Table 3 compares the MAO activity in organ homogenates prepared from normal mice with the MAO activity in organ homogenates prepared from obese and obese-DR mice. The MAO activity in the kidneys, testes, and white fat of obese mice and obese-DR mice was significantly greater than the MAO activity of the organs of normal mice. There was no significant difference in the MAO activity of liver, brain, brown fat, heart, or pancreatic islets in the three experimental groups of mice.

To determine if there was any correlation between the degree of obesity and the elevation in tissue MAO activity, we calculated the correlation coefficients (r) between body weight of the obese and obese-DR groups and the MAO activity in their kidneys and testes. The weight of the 16 mice in the analysis ranged from 24.0 to 51.0 gm. (mean 39.1 gm.). There was no significant correlation between body weight and kidney MAO activity ($r = -0.08$) or between body weight and testis MAO activity (-0.10).

Table 4 compares the NE content of kidney, white fat, and brown fat in normal, obese, and obese-DR mice. There was no significant difference in the NE content of each of these tissues in the three groups of mice. In addition, we attempted to measure the NE content of isolated pancreatic islets in two normal, two obese, and two obese-DR mice. An extract composed of 20 islets from each of the mice was present in the NE assay tube. The NE content of the mouse islets was below the sensitivity of the NE assay (less than 3 pg. per islet). In contrast to these low values, the mean NE content of pancreatic islets isolated from nine different golden hamsters was 75 ± 9 pg. per islet.

DISCUSSION

This report demonstrates that homogenates of kidney, testis, and white fat prepared from tissues of obese mice have greater MAO activity than homogenates of these organs prepared from tissues of normal mice. Mitochondrial-rich pellets prepared from kidneys of obese mice have greater MAO activity than those prepared from kidneys of normal mice. The increased MAO activity (Vmax) in kidneys from obese mice can be demonstrated by both kinetic analysis of data obtained by varying the concentration of tryptamine and by the use of a saturating concentration of tryptamine. The Km of kidney MAO for tryptamine is similar in normal and obese mice, suggesting that the increased MAO activity is due to a greater amount of tissue MAO rather than to an altered affinity of MAO for the tryptamine substrate. In contrast, the obese mice have significantly less COMT activity in their liver than the normal mice.

The increased MAO activity in the ob/ob mice may be a primary disturbance, or it may be secondary to obesity or hyperglycemia. This increased enzyme activity was not altered when the weight of the mice was decreased by chronic caloric restriction. Some members of this obese-DR group of mice had similar

TABLE 4

NE content of organs from normal, obese, and obese-DR mice

Tissue	NE (ng./mg. tissue protein)* Normal	Obese	Obese-DR
Kidney	1.94 ± 0.15	1.92 ± 0.28	2.15 ± 0.22
White fat	1.63 ± 0.52	1.97 ± 0.93	2.40 ± 0.92
Dark fat	2.32 ± 0.78	2.81 ± 0.84	2.76 ± 1.01

*Each value is Mean ± S.E.M. of eight mice. There is no significant difference in the NE content of each organ in the three groups.

weights to normal mice, and the mean weight of the obese-DR group was intermediate between that of the normal and obese groups. Despite this weight reduction, the MAO activity in the obese-DR mice was as high as (kidney) or even slightly higher than (testis, white fat) the MAO activity in the obese mice. The majority of the obese mice in the second series of experiments, which were older than the mice in the first series of experiments, had normal plasma glucose levels. Despite the absence of hyperglycemia, they still had elevated MAO activity in their kidneys, testes, and white fat. The persistent elevations in MAO activity in the obese-DR mice despite euglycemia and reduction in the degree of obesity suggests that the elevated MAO activity in ob/ob mice is a primary rather than a secondary disturbance.

Obese mice have an increased rate of pancreatic insulin secretion.[8] Inhibitors of insulin secretion such as NE, dopamine, and serotonin are present in the pancreatic islets of many species.[20] Prior to performing our study, we speculated that an increase in islet MAO activity in ob/ob mice might result in a decrease in the islet monoamine content. The decreased concentration of a naturally occurring inhibitor of insulin secretion might contribute to the increased insulin secretion of ob/ob mice. Our experiments demonstrated that there was no change in islet MAO activity in obese and obese-DR mice. The islet NE content of all three groups of mice was extremely low. Thus, changes in islet MAO do not play a role in the abnormal insulin secretion of ob/ob mice.

Finally, one might ask if the increase in MAO activity in white fat contributes to the abnormalities in fat metabolism of ob/ob mice. Epididymal adipose tissue obtained from fasted obese mice releases less free fatty acids (FFA) than does epididymal adipose tissue obtained from fasted normal mice.[21,22] Epididymal adipose tissue from obese mice exhibited an impairment in its ability to mobilize FFA when incubated with E.[21] Although these differences may be due to the decreased number of fat cells per volume of epididymal adipose tissue in obese mice,[22] it is conceivable they are due to increased MAO activity in adipose tissue of obese mice. The increased MAO could impair lipolysis by rapidly destroying lipolytic monoamines. In fact we found no alteration in the total NE content of white fat from obese and obese-DR mice. This does not completely rule out a role for altered NE levels in ob/ob mice, for Gey and Pletscher have suggested that the major function of MAO is the rapid inactivation of free, biologically active monoamines rather than regulation of the content of total tissue monoamines.[23] In addition, the MAO may regulate the level of a monoamine that was not measured in this study. Serotonin, a monoamine that also has lipolytic effects, is also present in substantial quantities in white adipose tissue;[24,25] perhaps the tissue levels of serotonin are decreased by the increased MAO activity.

ACKNOWLEDGMENTS

This work was supported by the Veterans Administration (2650-1) and a research grant from the American Diabetes Association.

We thank Ms. Joan M. Roche, Ms. Judith Blalock, and Ms. Lea B. Foster for their technical assistance and Ms. Bonnie Woodlief for her secretarial assistance.

REFERENCES

[1]Rath, R., Veselkova, A., Vidlakova, M., and Masek, J.: Excretion of vanilmandelic acid by obese women. Am. J. Clin. Nutr. *20*:457-61, 1967.

[2]Halter, J., and Porte, D., Jr.: Increased adrenergic activity in diabetes mellitus (DM): response to therapy and pharmacologic stimulation. Clin. Res. *25*:160A, 1977 (abstr.).

[3]Cryer, P. E., Silverberg, A. B., Santiago, J. V., and Shah, S. D.: Plasma catecholamines in diabetes: the syndromes of hypoadrenergic and hyperadrenergic postural hypotension. Diabetes *25* (Suppl. 1):347, 1976 (abstr.).

[4]Christensen, N. J.: Plasma catecholamines in long-term diabetics with and without neuropathy and in hypophysectomized subjects. J. Clin. Invest. *51*:779-87, 1972.

[5]Sharman, D. F.: The catabolism of catecholamines: recent studies. Br. Med. Bull. *29*:110-15, 1971.

[6]Ingalls, A. M., Dickie, M. M., and Snell, G. D.: Obese, a new mutation in the house mouse. J. Hered. *41*:317-19, 1950.

[7]Bray, G. A., and York, D. A.: Genetically transmitted obesity in rodents. Physiol. Rev. *51*:598-646, 1971.

[8]Stauffacher, W., Orci, L., Cameron, D., Burr, I. M., and Renold, A. E.: Spontaneous hyperglycemia and/or obesity in laboratory rodents: an example of the possible usefulness of animal disease models with both genetic and environmental components. Recent Prog. Horm. Res. *27*:41-95, 1971.

[9]Hellerstrom, C., Hellman, B., and Larsson, S.: Some aspects of the structure and histochemistry of the adrenals in obese-hyperglycemic mice. Acta Pathol. Microbiol. Scand. *54*:365-72, 1962.

[10]Westman, S.: Development of the obese-hyperglycemic syndrome in mice. Diabetologia *4*:141-49, 1968.

[11]Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes *16*:35-39, 1967.

[12]Feldman, J. M., and Chapman, B.: Preparation of islets of Langerhans from rabbits and hamsters by the collagenase digestion technique. Acta Diabetol. Lat. *3-4*: 208-18, 1975.

[13]Wurtman, R. J., and Axelrod, J.: A sensitive and specific

assay for the estimation of monoamine oxidase. Biochem. Pharmacol. *12*:1439-41, 1963.

[14]Dixon, M., and Webb, E. C.: Enzymes. New York, Academic Press, 1964, pp. 313-34.

[15]McCaman, R. E.: Microdetermination of catechol-O-methyltransferase in brain. Life Sci. *4*:2353-59, 1965.

[16]Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurements with the Folin phenol reagent. J. Biol. Chem. *193*:265-75, 1951.

[17]Henry, D. P., Starman, B. J., Johnson, D. G., and Williams, R. H.: A sensitive radioenzymatic assay for norepinephrine in tissues and plasma. Life Sci. *16*:375-84, 1975.

[18]Saifer, A., and Gerstenfeld, B.: Photometric micro determination of blood glucose with oxidase. J. Lab. Clin. Med. *5*:448-60, 1958.

[19]Zar, J. H.: Biostatistical Analysis. Englewood Cliffs, Prentice Hall, 1974.

[20]Cegrell, L.: The occurrence of biogenic monoamines in the mammalian endocrine pancreas. Acta Physiol. Scand. Suppl. *314*:1-26, 1968.

[21]Marshall, N. B., and Engel, F. L.: The influence of epinephrine and fasting on adipose tissue content and release of free fatty acids in obese-hyperglycemic and lean mice. J. Lipid Res. *1*:339-42, 1959-1960.

[22]Westmen, S., and Hellman, B.: Release of fatty acids from the isolated epididymal fat pad of obese-hyperglycemic mice. Med. Exp. *8*:193-99, 1963.

[23]Geÿ, K. F., and Pletscher, A.: Activity of monoamine oxidase in relation to the 5-hydroxytryptamine and norepinephrine content of rat brain. J. Neurochem. *6*:239-43, 1961.

[24]Carlson, L. A., Ekelund, L. G., and Oro, L.: Metabolic and cardiovascular effects of serotonin. Life Sci. *6*:261-71, 1967.

[25]Stock, K., and Westermann, E. O.: Concentration of norepinephrine, serotonin, and histamine, and of amine-metabolizing enzymes in mammalian adipose tissue. J. Lipid Res. *4*:297-304, 1963.