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The Effect of Hypothalamic Lesion in the Sand Rat Maintained on a High Fat Diet

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Summary. Nine sand rats maintained on a high fat diet gained weight, developed endogenous hyperinsulinaemia and resistance to exogenous insulin without further deterioration in glucose tolerance. A four hour post glucose hyperinsulinaemia, noted in some animals on both diets, may represent a chronic stress to the islets in these intermittent feeders. Five animals survived post lesion. Results were consistent with previous findings that lesions in the posterior median eminence portion of the periventricular arcuate system were associated with an improved glucose tolerance, and also in the present study, an increased sensitivity to exogenous insulin. A possible explanation for the mechanism of these effects on carbohydrate metabolism is suggested.

Effets des lésions hypothalamiques chez le rat des sables maintenu à un régime riche en graisses

Résumé. Neuf rats des sables soumis à un régime très riche en graisses ont pris du poids, montré une hyperinsulinémie endogène et une résistance à l' insuline exogène sans autre modification de la tolérance au glucose — Quelques animaux soumis aux deux régimes ont montré une hyperinsulinémie quatre heures après l'absorption de glucose, dont la signification pourrait être un stress des îlots dû à l'intermittence des prises alimentaires — Cinq animaux ont survécu après lésion — Les résultats confirment les précédents: les lésions de la partie postérieure de l'éminence médiane du périventricularis sont liées à une amélioration de la tolérance au glucose, ainsi, comme le montre cette étude, qu' à une augmentation de la sensibilité à l' insuline exogène. Une hypothèse sur les mécanismes de ces effets sur le métablisme des carbohydrates est proposée.

Die Auswirkung hypothalamischer Läsionen bei Sandratten unter einer fettreichen Diät

Zusammenfassung. Neun Sandratten nahmen unter einer sehr fettreichen Diät an Gewicht zu, entwickelten eine endogene Hyperinsulinämie und eine Resistenz gegenüber exogen zugeführtem Insulin, ohne daß es zu einer weiteren Verschlechterung der Glucosetoleranz kam. Die Hyperinsulinämie, wie sie 4 Std nach Glucosegaben bei einem Teil der Tiere unter beiden Diäten festgestellt wurde, könnte Ausdruck einer chronischen Belastung der Inseln bei diesen Tieren mit verteilter Nahrungsaufnahme sein. Fünf Tiere überlebten das Setzen der Läsionen. Die Ergebnisse stimmten mit früher erhobenen Befunden überein, daß Läsionen im Bereich der eminentia mediana posterior des periventrikulären Systems die Glucosetoleranz verbessern, wobei sie in der vorliegenden Untersuchung die Empfindlichkeit gegenüber exogen zugeführtem Insulin verstärkten. Für den Mechanismus dieser Effekte auf den KH'Stoffwechsel wird eine Erklärungsmöglichkeit vorgeschlagen.

Key-words: Sand rat, hypothalamic lesion, high fat diet, glucose tolerance.

Introduction

Previous studies have suggested an ameliorative effect of lesions in that part of the periventricular arcuate system situated in the median eminence on the diabetic glucose tolerance of the sand rat [12, 11, 10]. In this study, 9 animals were stressed with a high-fat diet and several parameters related to carbohydrate metabolism were evaluated pre and post lesion, with histologic correlation in the 5 surviving animals.

Materials and Methods

Details on care of animals and oral glucose tolerance procedures are described in a previous paper [10]. Each glucose tolerance test pooled results from 2 separate days (0′, 30, 120″, on one day and 15′, 60′, and 240′ on another day) with the exception of the baseline glucose tolerance, which was done on 4 separate days. Serum was collected for endogenous insulin determination at the time each blood sample was drawn for glucose analysis, except on the final glucose tolerance of the lesioned animals, when insulin was not measured. Insulin tolerance tests (ITT) were performed using 0.25 U of purified pork insulin per kg body weight (0.005% glucagon) except for the "baseline" test in one animal (SR 164 C), where 0.125 U/kg were injected because of fasting hypoglycaemia. "Baseline" studies were performed on the vegetable-fed animals, repeated on the high-fat diet and post lesion.

On 2/6/68 all animals were weaned to chow by a supplementation of Purina lab chow ad libitum with their regular vegetable diet. One month later, the chow was replaced with high-fat Old Guilford Breeder Pellets ad libitum, and this regimen was maintained throughout the remaining experimental period[1]. Animals were weighed periodically at about 9 AM in the fed state.

1 Composition of the Old Guilford Breeder Pellets (Guilford, Conn.):

Protein	19%	Gross Energy 4.4 kcal/g
Fat	11%	Utilizable energy 3.86 kcal/g
Fibre	2.7%	
NFE	52%	

Average daily caloric intake (ADCI) was calculated using a water-loss correction for the vegetables (40% carrots, 50% spinach).

4 weeks after the initiation of high-fat feeding the animals were lesioned using a Stoelting DC lesionmaker and a standard stereotaxic apparatus. A stainless steel electrode was used for placing the lesions. The stereotaxic coördinates were F = Bregma — 1.6, Vertical = Skull — 8.7 placed in the midline. A current of 1 milliampere was passed for 15 sec. Animals were anaesthetized with Avertin (25 mg/100 g rat I.P.).

At termination of the experiments, the animals were killed by rapid decapitation. Brains were fixed in 10% formalin, and histological material prepared by frozen section (50 μ thick) and subsequent staining with cresyl violet. Lesions were localized by metal deposition and histological examination. Histology was read blind (J.H.). Plasma insulin levels were measured by a sensitive radioimmunoassay [62] using a rat insulin standard, a pork insulin tracer and guinea pig antisera to pork insulin. Owing to the nature of the assay system, values are expressed in relative units, rather than in units of sand rat insulin.

Table 1. *Blood glucose values during glucose tolerance tests of sand rats maintained on two diets. Blood glucose values are expressed in mg % and time in minutes. Figures in parentheses are standard deviations. The figures shown had to be pooled from 2 GTT carried out on two separate days (samples 0, 30, 120 taken on one day, 15, 60, 240 on the other). Individual data are shown for 4 animals but 9 were fully tested in each group*

				GTT (Vegetable diet)					
S.R.#	Sex	Weight (grams)	Age (mos.)	0′	15′	30′	60′	120′	240′
28	M	187	22	70	155	216	144	107	54
70	F	193	26	63	122	165	182	135	57
174 B	M	132	11	59	119	178	229	147	48
219	F	107	11	62	99	67	157	24	58
Mean 9 animals		168(34)		62(4)	120(22)	155(42)	159(50)	120(50)	58(8)
				GTT (High fat diet)					
S.R.#		Weight (grams)	Age[a]	0′	15′	30′	60′	120′	240′
28		202		71	150	144	148	89	63
70		226		57	113	142	100	80	53
174 B		199		61	96	144	162	144	68
219		174		65	127	161	110	81	60
Mean 9 animals		204(25)		64(5)	125(17)	156(43)	137(33)	105(39)	66(9)

[a] 1 month later.

Table 2. *Plasma insulin of 9 sand rats during GTT's carried out while on a vegetable diet. Insulin values are expressed in relative units (μU/ml) and time in minutes. Figures in parentheses are standard deviations. Insulin determinations were performed on the samples for which glucose values are reported (Table 1, and Fig. 4, 6, 8 and 10). As for Table 1, the figures had to be pooled from results on two separate days*

	Endogenous Insulin (Vegetable diet)					
S.R.#	0′	15′	30′	60′	120′	240′
28	4	10	136	>300	122	4
70	4	11	24	30	110	10
174 B	1	2	6	2	6	2
219	3	2	1	3	1	1
164 C	3	22	52	72	6	4
85	10	20	122	78	96	32
91	10	52	>300	>300	>300	64
100	4	13	82	2	180	128
179	6	60	78	88	88	44
Mean:	5.0 (2.9)					32(42)

Table 3. *As Table 2, but GTT's carried out on animals on high-fat diet*

	Endogenous Insulin (High fat diet)					
S.R.#	0′	15′	30′	60′	120	240″
28	6	120	230	78	136	6
70	6	132	110	80	60	8
174 B	8	10	98	72	216	18
219	82	>300	>300	>300	>300	40
164 C	62	>300	>300	>300	62	98
85	10	150	>300	28	300	16
91	58	>300	300	240	>300	>300
100	8	132	18	118	300	88
179	8	78	48	54	98	36
Mean:	28(31)					>70

Results

Prelesion. While on the regular chow supplemented diet 7 of 9 animals gained weight, the average gain being 17 g (S.D. 20) on an average daily calory intake (ADCI) of 27 (S.D. 5) calories. On the high-fat diet,

all 9 animals gained additional weight, an average of 23 (S.D. 8) g per animal on an ADCI of 31 (S.D. 4) calories, until the time of lesioning. Insulin tolerance tests (Fig. 1) showed significant difference in the de-

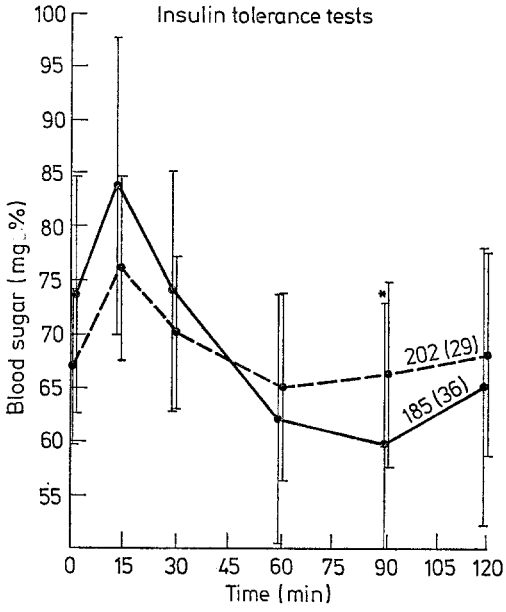


Fig. 1. Insulin tolerance tests for all 9 animals and on two diets. The data on the 5 surviving lesioned animals are also shown in Figs. 3, 5, 7, 9 and 11. ———— vegetable diet; — — — — high fat diet
[a] $P<0.02$.

cline of blood sugar from fasting levels to 90 min between the vegetable and fat fed groups in that fasting levels of the former were higher and those at 90 min lower than those of the fat-fed animals

While, in spite of an average gain in weight of 36 g per animal, glucose tolerance was not influenced by the high fat diet (Table 1), plasma insulin concentrations, both in the basal state and after glucose administration, were considerably higher after fat feeding (Tables 2 and 3). In addition, insulin secretion in response to hyperglycaemia appeared to be biphasic (diminution of insulin levels at 1 h (Table 3).

A sluggish early response of the islets to glucose is seen in all of the vegetable fed sand rats. In addition, in spite of comparable blood sugars, plasma insulin concentrations 4 h after glucose varied considerably in that they stayed elevated in some animals and had returned to basal levels in others.

Post lesion. Five of the nine animals survived for study. The ADCI was diminished in 4 of these animals from a prelesion average of 32 (S.D. 0.86) to 25 (S.D. 3.6) post lesion. In spite of this, 3 of them recovered their prelesion weights while SR 85 continued to lose weight. SR 179 gained weighed on the same ADCI of 29. Post mortem examination showed remarkable fat accumulation throughout the abdomen and subcutaneous tissues in all animals, including SR 85, which lost more than 25% of its body weight post lesion.

Two sand rats, 85 and 164 C, had lesions clearly posterior to the arcuate nucleus in the mammillary

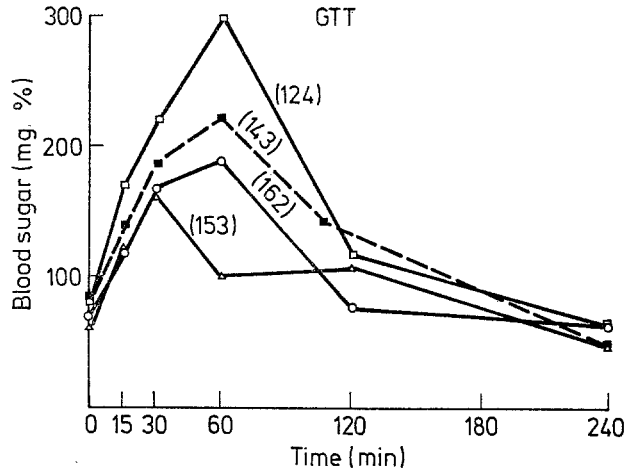


Fig. 2. GTT of rat 85. The figures in parentheses are weights.

△————△ Vegetable diet
○————○ 2½ weeks on high fat diet — prelesion
□————□ High fat diet, 4 weeks post lesion
■————■ High fat diet, 7 weeks post lesion

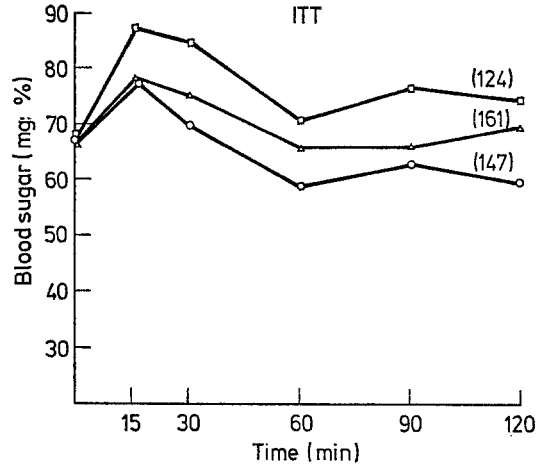


Fig. 3. ITT of rat 85. The figures in parentheses are weights.

△————△ Vegetable diet
○————○ 2½ weeks on high fat diet — prelesion
□————□ High fat diet, 5 weeks post lesion
■————■ High fat diet, 7 weeks post lesion

bodies. Neither showed an improvement in insulin tolerance and the glucose tolerances deteriorated post lesion, (Figs. 2—5). SR 91 also had a posterior lesion mostly in the mammillary area but extending into the tail end of the arcuate nucleus. This animal behaved

like animals 85 and 164 C with no response in glucose and little in insulin tolerance (Figs. 6, 7). SR 100 had a lesion extending from the premammillary area into the mid-portion of the arcuate nucleus, that is somewhat anterior to the lesion in animal 91. This rat remained normoglycaemic and demonstrated an increased sensitivity to exogenous insulin (Figs. 8, 9). SR 179 with a lesion in the mid-portion of the arcuate nucleus extending into the ventromedial nucleus unilaterally had an improvement in glucose and insulin tolerance despite continued weight gain (Figs. 10, 11).

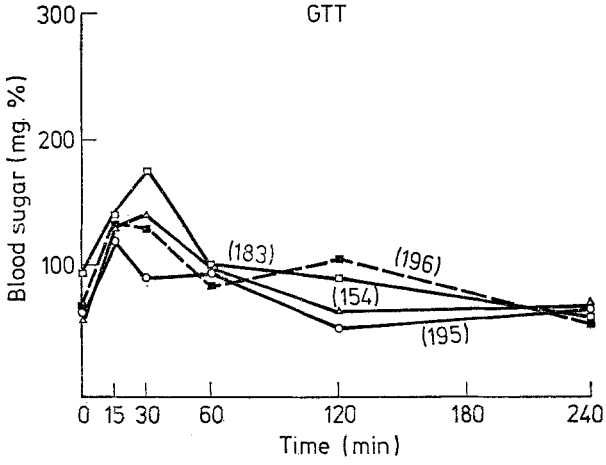


Fig. 4. GTT of rat 164 C. Data shown as in Fig. 2

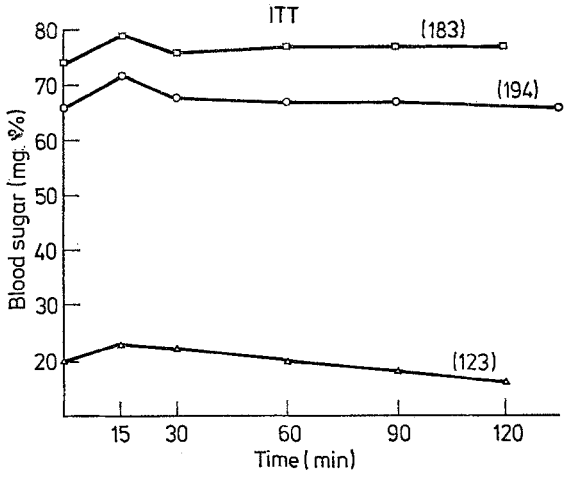


Fig. 5. ITT of rat 164 C. Data shown as in Fig. 3

No distinctive pattern of endogenous insulin response was seen post lesion, although all four hyperinsulinaemic animals had lower values. With the exception of animal 85 which had lost considerable weight, the sand rats demonstrated a persistent post lesion hyperinsulinaemia 4 h after oral glucose.

Discussion

Prelesion. In the sand rat, a high fat diet appeared to induce weight gain, resistance to exogenous insulin, and hyperinsulinaemia without a further deterioration in the glucose tolerance during the period of this study.

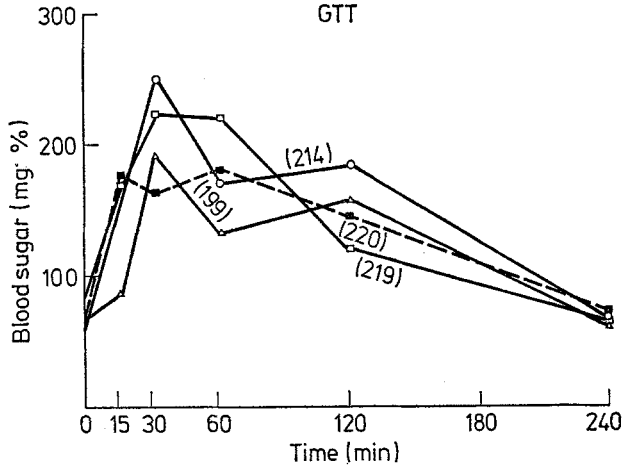


Fig. 6. GTT of rat 91. Data shown as in Fig. 2

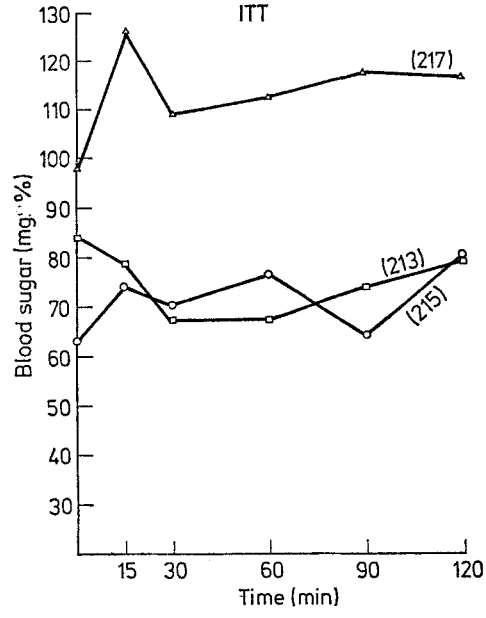


Fig. 7. ITT of rat 91. Data shown as in Fig. 3

Although insensitivity to exogenous insulin has also been reported in studies on the white rat maintained on either a 12% or 40% fat diet, hyperglycaemia, lower plasma insulin levels and a diminished secretory re-

sponse of the beta cells to glucose have been noted in these animals [44, 6]. In a similar study in the Wellesley hybrid mouse, however, hyperglycaemia and hyperinsulinaemia have been reported [31]. The reasons for these discrepancies are unknown but may be related to differences in the composition of the diets used.

that lead to a 4 h post-glucose hyperinsulinaemia in these intermittent feeders may represent a chronic metabolic stress to the islets.

Judging from our data on the glucose tolerance and endogenous plasma insulin response in the sand rat, and the lack of correlation between these parameters

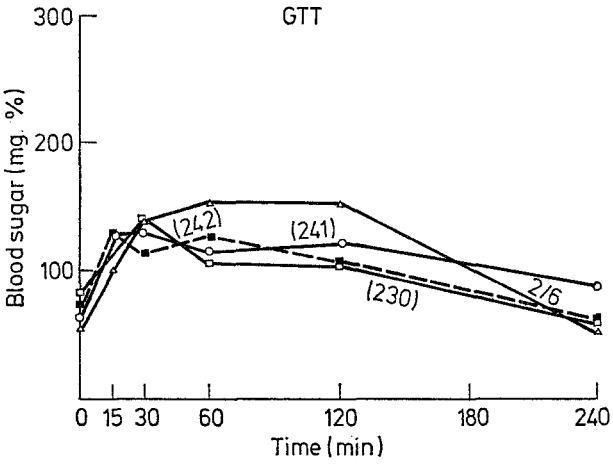


Fig. 8. GTT of rat 100. Data shown as in Fig. 2

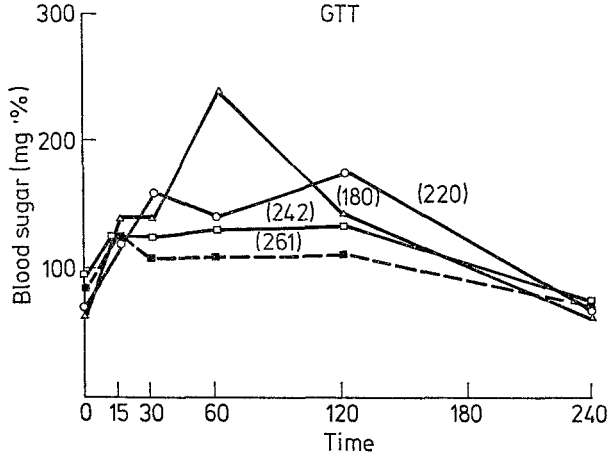


Fig. 10. GTT of rat 179. Data shown as in Fig. 2

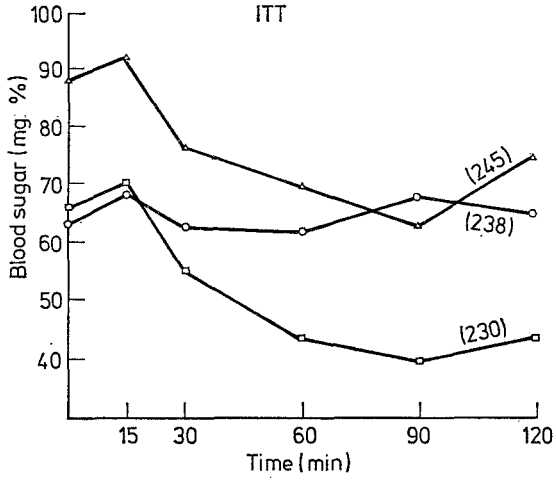


Fig. 9. ITT of rat 100. Data shown as in Fig. 3

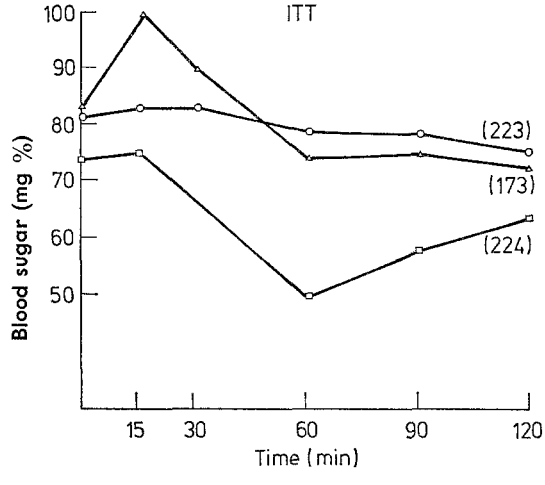


Fig. 11. ITT of rat 179. Data shown as in Fig. 3

Fasting and 4 h blood sugar levels were comparable on both diets, but relative hyperinsulinaemia was noted 4 h after glucose administration in some animals on either diet. The chow induced diabetic syndrome in the sand rat is characterized by hyperinsulinaemia at first, and then low plasma and pancreatic insulin content in its advanced stages [34, 45]. The conditions

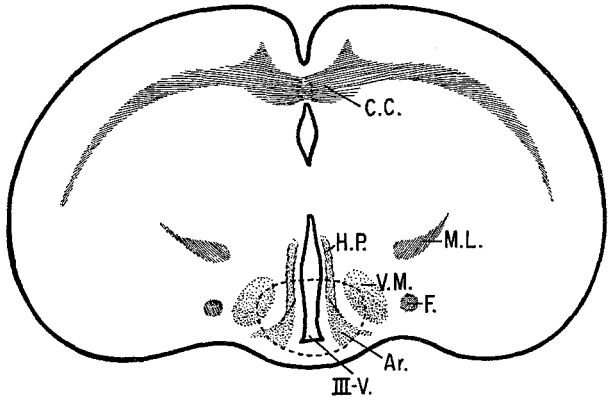
(Tables 1—4), the plasma insulin concentration does not appear to be the sole or possibly even the principal determinant of normoglycaemia in this species.

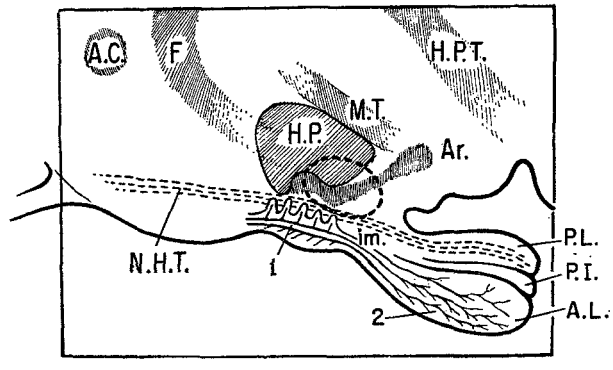
Also of interest is the sluggish early response of the islets to a glucose stimulus seen in all of the vegetable-fed sand rats, a finding first reported in human diabetics in 1960 [62]. It is known that sympathetic type

neurotransmitters are associated with the aldehyde fuchsin granules of the islets in some species [23], and that an adrenergic stimulation can impair glucose-mediated insulin release [18, 48, 25]. In addition, a Somogyi effect associated with a diminished early insulin response has recently been reported in normal humans 4—6 h after a glucose load [63], and appears to depend on an intact anterior pituitary and to be related in time to the secretion of growth hormone in response to a fall in blood sugar. Whether or not mechanisms of this type play a significant role in the responsiveness of the sand rat islets to glucose is unknown at present.

Table 4. *As Tables 2 and 3, but carried out on lesioned animals*

	Post lesion endogenous insulin (High fat diet)					
S.R.#	0′	15′	30′	60′	120′	240′
164 C	10	180	>300	40	52	180
85	8	140	58	6	128	14
91	24	>300	150	80	30	174
100	4	180	10	10	12	80
179	8	120	160	30	16	64





Figs. 12 and 13

A.C. — Anterior commissure
A.L. — Anterior lobe pituitary
Ar. — Arcuate nucleus
F. — Fornix
H.P. — Periventricular extension of arcuate nucleus
H.P.T. — Habenulopeduncular tract
im. — infundibulum
M.T. — Mammilothalamic tract
N.H.T. — Neurohypophyseal tract
P.I. — Intermediate lobe pituitary
P.L. — Posterior lobe pituitary
1 — Primary plexus
2 — Secondary plexus
c.c. — Corpus callosum
V.M. — Ventromedial nucleus
M.L. — Medial lemniscus
III—V — Third ventricle
— — — — Site of effective lesions

That the islets of the sand rat can respond to a metabolic stress, however, is evidenced by the elevated insulin concentrations and their rapid rise after glucose administration in fat-fed animals, in which an increased intake of carbohydrate, fat and protein leads to the development of obesity.

Post lesion. Amelioration of pancreatic diabetes after hypothalamic injury in cats was noted in 1935 [19], but not in partially depancreatized rats in a subsequent study [9]. From the present and past studies on the sand rat [12, 11, 10], it would appear that lesions in the posterior median eminence extending into the arcuate nucleus in the region of the ventromedial nuclei (Figs. 12, 13) improve the glucose tolerance independent of weight loss.

An improvement in insulin sensitivity has been reported after hypothalamic lesioning in several species [59, 40, 14, 41, 39], but not confirmed in cats [8]. Also of note in the present study is the increased sensitivity to exogenous insulin seen in the two sand rats with lesions in the arcuate system. That this increased sensitivity occurred in the presence of marked obesity suggests that insulin resistance in this species results not only from adiposity, but that an additional influence emanating from the hypothalamus is equally important.

The four hyperinsulinaemic animals had diminished endogenous insulin levels post lesion. Whether this was due to compensation after the prolonged stress of the high fat diet or to central neurogenic effects produced by the lesion is still unknown; the same holds true for the mechanisms responsible for the maintenance or increase of weight observed in 4 of the animals despite reduced or isocaloric intakes, as physical activity, water balance, heat production, body composition and intestinal absorption of foodstuffs were not measured. Studies in other species suggest that diminished thermogenesis is an important factor in the altered energy metabolism of the lesioned animal [13, 7, 35].

How these median eminence lesions influence carbohydrate metabolism also remains to be explained. A role for the central nervous system in the control of carbohydrate metabolism was first suggested by Claude Bernard in 1858, and autonomic control mechanisms apparently have an influence on hepatic [26, 57, 56, 55], adipose tissue [60, 37, 53, 33], adrenal medullary [22, 32] pituitary [5, 29] and islet cell [23, 18, 48, 25, 30, 28, 24, 64] function, although the influence of the vagus on immunoreactive insulin release in dogs [28] was not confirmed [47]. The median eminence

may be involved in the elaboration of rat GHRF [42, 51], and this general area also appears to be involved in the secretion of growth hormone occurring after insulin hypoglycaemia in the monkey [1]. Several reports have suggested a role for the ventromedial nucleus (VMN) in the elaboration and/or secretion of growth hormone [38, 36, 4, 27, 50]. The VMN, although bilateral, lies in close proximity to and extends alongside the mid portion of the arcuate nucleus, which is considered by some to be the final common neuro-endocrine path [49]. In a recent study bilateral lesions of the VMN were associated with a reversion toward normal of hyperglycaemic blood sugar values in another diabetic species, the *db* mouse [16]. Although the central neural connections of the sympathetic nervous system have not been clearly defined, earlier studies have suggested a component of this central pathway in the ventral and median area of the hypothalamus [43, 3], and a role for this area in gluconeogenesis and glycogenolysis has been reported [26, 57]. Evidence for insulin sensitivity of the satiety centre was first presented in 1964 [2]. Recent studies have confirmed this observation and provided additional evidence that the oligodendrocytes are the specific glucoreceptors of the VMN and are responsive to insulin [21, 20]. It is possible that insulin resistance in these cells may trigger diabetes in the whole animal by potentiating central neural stimuli for glucose provision during starvation, and preventing or inhibiting their shut off during hyperglycaemia [58, 46, 29, 17, 61, 15, 54]. Lesions in the posterior median eminence may influence carbohydrate metabolism by interrupting these reflex pathways.

The results of this study, taken in conjunction with our previous reports [12, 11, 10], suggest that posterior median eminence lesions in the area of the arcuate system have an ameliorative effect on the diabetic glucose tolerance of the sand rat, and also improve insulin sensitivity. Further studies are needed to clarify the mechanism of this effect.

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References

1. Abrams, R.L., Parker, M.L., Blanco, S., Reichlin, S., Daughaday, W. H.: Hypothalamic regulation of growth hormone secretion. Endocrinology **78**, 605 (1966).
2. Anand, B.K., Chhina, G.S., Sharma, K.N., Dua, S., Singh, B.: Activity of single neurons in the hypothalamic feeding centers. Effects of glucose. Amer. J. Physiol. **207**, 1146—1154 (1964).
3. Beattie, J.: Hypothalamic mechanisms. Canad. med. Ass. J. **26**, 400 (1932).
4. Bernardis, L. L., Skelton F. R.: Growth and obesity following ventromedial hypothalamic lesions placed in female rats at four different ages. Neuroendocrinology **1**, 265 (1966).
5. Blackard, W.C., Heidingsfelder, S.A.: Adrenergic receptor control mechanism for growth hormone secretion. J. clin. Invest. **47**, 1407 (1968).
6. Blazquez, E., Lopez Quijada, C.: The effect of a high-fat diet on glucose, insulin sensitivity and plasma insulin in rats. J. Endocr. **42**, 489—494 (1968).
7. Brobeck, J.R.: Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol. Rev. **26**, 541—559 (1946).
8. — Insulin sensitivity of cats with hypothalamic lesions and cats with cervical cord section. J. Lab. clin. Med. **25**, 717—725 (1940).
9. — Tepperman, J., Long, C.N.H.: The effect of experimental obesity upon carbohydrate metabolism. Yale J. biol. Med. **15**, 893—904 (1943).
10. Brodoff, B.N., Zeballos, G.: Further studies on the effect of hypothalamic lesions in the sand rat. Diabetologia **6**, 366—370 (1970).
11. — — Dorn, J.: A possible role for the central nervous system in the sand rat diabetes. Excerpta med. int. Congress Series No. 172. Proceedings of the VIth Congress of the Int. Diabetes Federation: 823, (1967).
12. — — — Amelioration of the diabetic glucose tolerance of the sand rat after hypothalamic injury. Metabolism **6**, 744 (1967).
13. Brooks, C.McC., Marine, D.N.: A study of oxygen consumption in obesity. Fed. Proc. **5**, 12 (1946).
14. Bryant Benson: Insulin sensitivity and adrenocortical function in guinea pigs with hypothalamic lesions: Acta endocr. **53**, 663—672 (1966).
15. Chang, A.Y., Schneider, D.I.: Abnormalities in hepatic enzyme activities during development of diabetes in db mice. Diabetologia **6**, 274—278 (1970).
16. Coleman, D.L., Hummel, K.P.: The effects of hypothalamic lesions in genetically diabetic mice. Diabetologia **6**, 263—267 (1970.
17. — — Studies with the mutation, diabetes, in the mouse. Diabetologia **3**, 238—248 (1967).
18. Coore, H.G., Randle, P.J.: Regulation of insulin secretion with pieces of rabbit pancreas incubated, *in vitro*. Biochem. J. **93**, 66 (1964).
19. Davis, L.E., Cleveland, D., Ingram, W.R.: Carbohydrate metabolism. The effect of hypothalamic lesions and stimulation of the autonomic nervous system. Arch. Neurol. Psychiat. **33**, 592—615 (1935).
20. Debons, A.F., Krimsky, I., From, A., Clautier, R.J.: Site of action of gold thioglucose in the hypothalamic satiety center. Amer. J. Physiol. **219**, 1397—1402 (1970).
21. — — — A direct action of insulin on the hypothalamic satiety center. Amer. J. Physiol. **219**, 938—943 (1970).
22. Duner, H.: The influence of the blood glucose level on the secretion of adrenaline and nor-adrenaline from the suprarenal. Acta physiol. scand. **28**, Suppl. 102 (1953).
23. Falck, B., Owman, C.: 5 Hydroxytryptamine and unrelated amines in endocrine cell systems. Advances in Pharmacology Vol. 6A p. 211—233. New York, London: Academic Press 1968.

24. Feldman, J.M., Lebovitz, H.E.: Inhibition of insulin secretion by serotonin. Abstracts American Diabetes Assoc. 29th Annual Meeting, 326 (1969).

25. — — Inhibition of insulin secretion by serotonin. Abstracts American Diabetes Assoc. 29th Annual Meeting, 326 (1969).

26. Friedman, N., Wertheimer, H.E.: A study of gluconeogenesis by the administration of 2 - Deoxyglucose. Metabolism **15**, 222—235 (1966).

27. Frohman, L.A., Bernardis, L.L.: Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. Abstract. Endocrinology **82**, 1125 (1968).

28. — Ezdinli and Rouhollah, J.: Effect of vagotomy and vagal stimulation on insulin secretion. Diabetes Vol. **16**, 443—448 (1967).

29. Gale, C.C., Toivola, P., Werrbach, J.H., Goodner, C.J.: Further studies of adrenergic mechanisms mediating reciprocal release of growth hormone and insulin in baboons. Fed. Proc. **29**, 377 (1970).

30. Gentes, M.: Note sur les terminaisons nerveuses des ilots de Langerhans du pancreas. C.R. Soc. Biol., Paris **54**, 202—203 (1902).

31. Gleason, R.E., Lauris, V., Soeldner, J.S.: Studies on experimental diabetes in the wellesley hybrid mouse. III. dietary effects and similar changes in a commercial swiss-hauschka strain. Diabetologia **3**, 175—178 (1967).

32. Goldfien, A.: Effects of glucose deprivation on the sympathetic outflow to the adrenal medulla and adipose tissue. Pharmacol. Rev. **18**, 303 (1966).

33. Goodner, C.J., Tustison, W.A., Davidson, M.B., Chu, P., Conway, M.J.: Studies of subtrate regulation in fasting.Diabetes **16**, 576—589 (1967).

34. Hackel, D.B., Frohman, L.A., Mikat, E., Lebovitz, H.E., Schmidt-Nielsen, K., Kinney, T.D.: Effect of diet on the glucose tolerance and plasma insulin levels of the sand rat (Psammomys obesus). Diabetes **15**, 105—114 (1966).

35. Han, P.W.: Energy metabolism of tube fed hypophysectomized rats bearing hypothalamic lesions. Amer. J. Physiol. **215**, 1343—1350 (1968).

36. — Lin, C.H., Chu, J.Y., Mu, J.Y., Liu, A.C.: Hypothalamic obesity in weanling rats. Amer. J. Physiol. **209**, 627 (1965).

37. Havel, R.J., Godlfien, A.: The role of the sympathetic nervous system in the metabolism of free fatty acids. J. Lipid Res. **1**, 102 (1959).

38. Hetherington, A.W., Ranson, S.W.: Hypothalamic lesions and adiposity in the rat. Anat. Rec. **78**, 149 (1940).

39. Ingram, W.R., Barris, R.W.: Evidence of altered carbohydrate metabolism in cats with hypothalamic lesions. Amer. J. Physiol. **114**, 562—571 (1936).

40. Kokka, N., George, R.: Electrical stimulation and lesions of the hypothalamus in alloxan diabetic rabbits. Brain Research **1**, 355—362 (1966).

41. — — Modification of insulin response and alloxan diabetes by hypothalamic lesions in rats. Neuroendocrinology **4**, 333—346 (1969).

42. Krulich, L., Dharwol, A.P.S., McCann, S.M.: Hypothalamic control of growth hormone (GH) secretion. Abstracts Annual Meeting of the Endocrine Society, 21 (1965).

43. Kurotsu, T.: Experimental and histological studies on the autonomic centers. Med. J. Osaka Univ. **1**, 8 (1949).

44. Malaisse, W.J., Lemmonnier, D., Malaisse-Lagae, F., Mandelbaum, Israel M.: Secretion of and sensitivity to insulin in obese rats fed a high fat diet. Horm. Metab. Res. **1**, 9 (1969).

45. Miki, E., Like, A.A., Soeldner, J.S., Steinke, J., Cahill, G.F., Jr.: Acute ketotic-type diabetic syndrome in sand rats (Psammomys obesus) with special reference to the pancreas. Metabolism **15**, 749—760 (1966).

46. Muller, W.A., Parada, E., Eisentraut, A., Unger, R.H.: Glucagon responses to food: abnormal a-cell function in diabetics. Clin. Res. Vol. **18**, 74 (1970).

47. Nelson, N.C., Blackard, W.G., Cocchiara, J.C., Labat, J.A.: Influence of the vagus nerves on pancreatic insulin secretion. Diabetes **16**, 852—857 (1967).

48. Porte, D. Jr., Graber, A., Kuzuya, T., Williams, R.H.: The effect of epinephrine on immuno-reactive insulin levels in man. J. clin. Invest. **45**, 228 (1966).

49. Reichlin, S.: Neuroendocrinology. Medical progress. New Engl. J. Med. **269**, 1182 (1963).

50. — Functions of the median eminence gland. NEJM **275**, 600 (1966).

51. — Growth and the hypothalamus. Endocrinology **67**, 760 (1960).

52. Sandler, R., Herrera, E., Freinkel, N.: Glucose overproduction in obese hyperglycemic mice. Clin. Res. **16**, 351 (1968).

53. Schotz, M.C., Page, I.H.: Effect of adrenergic blocking agents on the release of free fatty acids from rat adipose tissue. J. Lipid Res. **1**, 466 (1960).

54. Seidman, I., Horlandand, A.A., Teibor, G.W.: Glycolytic and gluconeogenic enzyme activity in the hereditary obese-hyperglycemic syndrome and in acquired obesity. Diabetologia **6**, 313—316 (1970).

55. Shimazu, T.: Glycogen synthetase activity in liver: Regulation by autonomic nerves, Science **156**, 1256—1257 (1967).

56. — Fukuda, O., Tadayasu, B.: Reciprocal influences of the ventromedial and lateral hypothalamic nuclei on blood glucose level and liver glycogen content: Nature **210**, 1178—1179, (1966).

57. — — Increased activities of glycogenolytic enzymes in liver after splanchnic-nerve stimulation. Science **154**, 1607—1608 (1965).

58. Sonksen, P.H., Gleason, R.E., Soeldner, J.S.: Abnormal serum growth hormone responses in genetic prediabetic male patients. Diabetes **18**, Suppl. **1**, 334 (1969).

59. Spirtos, B.N., Halmi, N.S.: Increased insulin sensitivity in rats with hypothalamic lesions. Endocrinology **65**, 669 (1959).

60. Wertheimer, E., Shapiro, B.: The physiology of adipose tissue. Physiol. Rev. **28**, 451 (1948).

61. Willms, B., Ben-Ami, P., Soling, H.D.: Hepatic enzyme activities of glycolysis and gluconeogenesis in diabetes of man and laboratory animals. Horm. Metab. Res. **2**, 135—141 (1970).

62. Yalow, R.S., Berson, S.A.: Immunoassay of endogenous plasma insulin in man. J. clin. Invest. **39**, 1157 (1960).

63. — Goldsmith, S.J., Berson, S.A.: Influence of physiologic fluctuations in plasma growth hormone on glucose tolerance. Diabetes **18**, 402—408 (1969).

64. Zunz, E., La Barre, J.: Sensibilité des centres nerveux superieurs à l'hyperglycemie provoquée par injection de dextrose. Compt. Rend. Soc. Biol. **96**, 1400—1403 (1927).

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