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18. Allende chromite has some 30 mole percent $Fe^{3+}$ in the $M^{3+}$ sites [D. Virgo, private communication cited in (*5*)], and the chromite of unequilibrated ordinary chondrites also seems to contain small amounts of $Fe^{3+}$, judging from the increase of Fe/Cr with PMD (*16*); see also Table 1.
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21. Little information exists on heats of solution of noble gases in solids; the most relevant data are those of M. S. Lancet and E. Anders [*Geochim. Cosmochim. Acta* **37**, 1371 (1973)] on magnetite at 450° to 700°K. Their heats of solution for Ne and Ar were −2.2 and −15.2 kcal/mole. Although heats of solution might vary from mineral to mineral, because of differences in size of lattice vacancies and bond strength, the difference between light and heavy gases should persist, because it depends mainly on the atomic properties of the gases themselves.
22. For an estimate for the Q content, we used the weight loss in the first $HNO_3$ treatment. Unfortunately, $HNO_3$ does not give a clean separation of Q and chromite; some chromite dissolves in the first treatment, whereas some Q survives. And since some of the samples weighed less than 2 mg, weighing errors are appreciable. However, estimates based on soluble Fe (Table 1) give fairly similar results, and the uncertainties are not large enough to affect our conclusions. The trend in Fig. 3 would have been essentially similar if we had merely plotted the gas contents per gram of residue (that is, assumed constant Q contents).
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# Somatostatin: Widespread Abnormality in Tissues of Spontaneously Diabetic Mice

Abstract. *Diabetic mice of the C57BL/6J obob and C57BL/Ks dbdb strains show a reduction in pancreatic somatostatin concentration accompanied in the obob strain by a striking decrease in the number of somatostatin-containing cells in the islets. Somatostatin concentration is also decreased in the stomach but increased in the hypothalamus. These findings suggest different control mechanisms for somatostatin in the hypothalamus compared to the gut and pancreas and exclude a primary genetic abnormality of somatostatin cells in the mutants.*

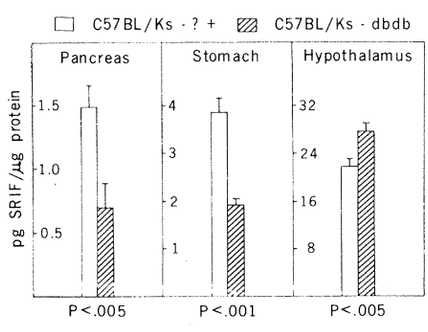
The obob and dbdb mice are single gene mutants characterized by obesity and hyperphagia. Mice of both strains develop spontaneous diabetes with different clinical features depending on the genetic background (*1*). Although the primary defect in the mutant mice has not been elucidated, substantial evidence exists for an underlying hypothalamic abnormality. Thus, apart from the abnormal appetite control which in the dbdb mouse is presumed to be secondary to a defect in the hypothalamic satiety center (*2*), the obob mouse in particular exhibits a variety of other dysfunctions suspected of being hypothalamic in origin, such as impaired thermoregulation, thyroid function, reproductive function, and growth hormone (GH) secretion (*3*).

Recent studies have revealed a striking reduction in the pancreatic concentration of immunoreactive somatostatin in 3-month-old obob and dbdb mice (*4*). Since somatostatin is widely distributed throughout the brain and gastrointestinal tract (*5, 6*), our study was undertaken to examine somatostatin in other organs. It demonstrates striking changes in the concentration of the peptide in the stomach and hypothalamus in addition to those of the pancreas of the obob and dbdb mice.

Male C57BL/6J obob and lean littermate controls C57BL/6J?+ were purchased from the Centre de Sélection et Élevage d'Animaux de Laboratoires, Orléans, France, and studied at 8 weeks of age. Male C57BL/Ks dbdb and lean controls C57BL/Ks?+ were obtained from the Jackson Laboratory, Bar Harbor, Maine, and studied at 11 to 12 weeks of age. Animals in the fed state were killed by decapitation, and trunk blood was collected for glucose and insulin estimations (*7*). The following tissues were extracted for somatostatin: whole hypothalamus and pyloric antrum from all mice (*8*), whole pancreas from the dbdb and control mice, and pancreatic islets from the obob and control mice. From a second group of obob and control mice, the whole pancreas was removed, divided longitudinally into two halves, one of which was extracted; the other half was fixed in Bouin's solution, sectioned, stained by immunofluorescence for somatostatin, and subjected to morphometric analysis.

Somatostatin was extracted from tissues by sonification in 1*N* acetic acid at 0°C and measured by specific radioimmunoassay (*4, 6*). The total protein concentration of the extracts was determined by the method of Lowry *et al.* (*9*).

For morphometric analysis, the somatostatin-containing cells were identified by indirect immunofluoresence and quantified in the first 20 islets encountered in sections of each pancreas. The volume density per islet of the somatostatin cells was determined by the point counting method of Weibel (*10*). In addi-



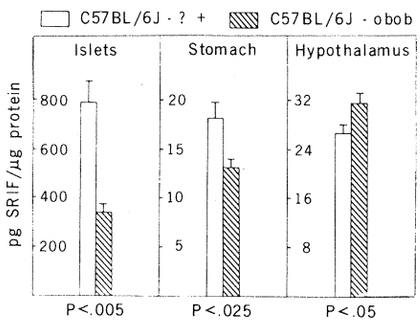




Fig. 1 (left). Comparison of somatostatin concentration in whole pancreas, stomach, and hypothalamus of dbdb ($N = 10$) and control ($N = 10$) mice. Means ± standard errors are plotted. Fig. 2 (right). Comparison of somatostatin concentration in islet, stomach, and hypothalamus of obob ($N = 10$) and control ($N = 10$) mice. The points represent means ± the standard errors.

tion, total islet volume and total cell volume for each pancreas were also calculated.

As was expected, the mutant mice were grossly obese compared to their lean controls and exhibited hyperglycemia and marked hyperinsulinemia. Pancreatic somatostatin concentration in the dbdb mice was reduced by 50 percent (Fig. 1). In the obob mice, the concentration of somatostatin in the whole pancreas was reduced by 25 percent, but this difference was not significant; in isolated pancreatic islets from the same animals, a 60 percent reduction in the somatostatin concentration was found (Fig. 2). Morphometric analysis (Table 1) of the obob pancreases revealed an increase in the pancreatic endocrine cell volume together with a reduction in the volume density of somatostatin immunofluorescent cells per islet of the diabetic mice. There was no significant change in total volume of somatostatin cells per pancreas. The reduction in somatostatin concentration in the pancreas and islets of the dbdb and obob mice was accompanied by a similar reduction in the somatostatin concentration of the stomach of both mutant mice (Figs. 1 and 2). By contrast, however, the concentration of somatostatin in the hypothalamus of the diabetic animals was significantly increased (Figs. 1 and 2).

The reduction of total pancreatic somatostatin concentration in the C57BL/Ks dbdb in this study is similar to our previous findings (*4*). Unlike our earlier results in a slightly older group of C57BL/6J obob mice from Bar Harbor, total pancreatic somatostatin concentration in the obob in the study reported here was not significantly decreased. Isolated islets from these animals, however, showed a reduction in the somatostatin concentration and in volume density of somatostatin-containing cells. Baetens *et al.* (*11*) have reported that both obob and dbdb genes in C57BL/6J mice are associated with a decrease in volume density of somatostatin-containing cells. Whether there is a similar reduction in somatostatin cells in the islets as opposed to the whole pancreas of young C57BL/Ks dbdb mice is unknown, although an increase in somatostatin cells has been noted in older animals lacking insulin (*11*).

Our study suggests that the somatostatin changes in the pancreas of the mutant mouse are not confined to this organ but are part of a more generalized abnormality affecting somatostatin in other organs as well. Thus, in both the obob and dbdb mice, the concentration of somatostatin in the stomach was reduced to approximately the same extent as in the whole pancreas, whereas it was increased in the hypothalamus.

Table 1. Volume of pancreas and endocrine pancreas and volume and number of somatostatin immunofluorescent cells per islet and per total pancreas in obob and control mice.

| Mice | *N* | Pancreas ($mm^3$) | Endocrine pancreas ($mm^3$) | Somatostatin cells | |
|---|---|---|---|---|---|
| | | | | Volume per pancreas ($mm^3$) | Volume density per islet |
| C57BL/6J?+ | 5 | 248 ± 10 | 1.3 ± 0.1 | 0.044 ± 0.008 | 3.5 ± 0.5 |
| C57BL/6J obob | 5 | 254 ± 9 | 5.0 ± 0.6* | 0.050 ± 0.013 | 1.1 ± 0.3† |

*$P$ = .001. †$P$ = .01.

The mechanisms responsible for the somatostatin changes remain unclear. The pattern of the tissue changes observed in the mutant mice contrasts sharply with that reported in rats with streptozotocin-induced diabetes, where there is a marked augmentation in the content of somatostatin in the islets, accompanied by an increase in the number of somatostatin-producing cells, and an increase in gastric somatostatin concentration but no change in hypothalamic somatostatin (*12, 13*). These findings imply a common secretory mechanism for the somatostatin cells in the pancreas and gut. The most striking hormonal difference between the obob and dbdb mutants on the one hand and the streptozotocin diabetic animals on the other is the marked hyperinsulinemia present in the former suggesting a reciprocal relationship between circulating insulin and somatostatin concentration in tissue.

It is conceivable that high circulating insulin suppresses the somatostatin-producing cells in the islets and in the gut by a negative feedback mechanism. Such a mechanism would also explain the reversal of the somatostatin changes in the older C57BL/Ks dbdb mice which have been reported to show an increase in volume density of somatostatin cells in the islets when they cease to produce insulin (*11*). The high concentration of somatostatin in the hypothalamus of the diabetic mutants is presumably a manifestation of the underlying hypothalamic abnormality in these animals since hypothalamic somatostatin concentration is unaltered in rats with streptozotocin-induced diabetes (*12*).

Since an alteration of somatostatin concentration in a particular organ could occur in states of either increased or decreased secretion, the functional significance of the somatostatin changes reported here remain uncertain. In the pancreas of the obob, however, the combination of a decrease in the islet content and in volume density of somatostatin-producing cells suggests a decrease of islet somatostatin function which may in part account for the hyperinsulinemia and hyperglucagonemia reported in these animals (*14*). The increased hypothalamic somatostatin concentration, if accompanied by increased secretion could explain the diminished GH secretion and thyroid function in these animals (*3*).

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