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Source: Journal of Wildlife Diseases, 20(3) : 253-255

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-20.3.253>

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## Spontaneous Diabetes Mellitus in a Captive Golden-mantled Ground Squirrel, *Spermophilus lateralis* (Say)

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Spontaneous diabetes mellitus has been reported in numerous domestic and wild mammals (Meier, 1960, *Diabetes* 9: 485-489; Renold, 1968, *Adv. Metab. Disord.* 3: 49-84; Stuhlman et al., 1977, *Lab. Anim. Sci.* 27: 477-481). However, among ground squirrels, spontaneous diabetes has been only reported for the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus* (Mitchill) (Stuhlman et al., op. cit.). This study reports a case of spontaneous diabetes mellitus in a captive golden-mantled ground squirrel.

During July-August 1974 several golden-mantled ground squirrels were live-trapped at the Santa Fe Ski Basin, 3,203 m elevation, Santa Fe County, New Mexico. These animals were kept subsequently in the Basic Animal Services Unit at the University of Arkansas at Little Rock at 21-22°C and 40-60% relative humidity with 12-15 air changes per hour. A 12 hr light-dark cycle was maintained. The squirrels were individually housed in plastic cages (20 × 25 × 48 cm) with aspen bedding. They were fed a commercial rodent diet (Purina Formulab 5008-C, Ralston Purina Co., Checkerboard Sq., St. Louis, Missouri 63199, USA) *ad libitum*; water was supplied in individual glass bottles *ad libitum*.

In June 1978 one of the animals was observed to be exhibiting polydipsia and polyuria. The animal was subsequently monitored over a 20-day period for water intake and urine production. This monitoring period was of long enough duration

to establish set patterns. During this time the urine was tested daily with Labstix Reagent Strips for Urinalysis (Ames Division, Miles Laboratories, Elkhart, Indiana 46515, USA) for glucose, protein, ketones and pH. At the end of the period, the animal was fasted for 24 hr and then killed by ether anesthesia and exsanguination by way of cardiac puncture. The blood was tested for D-glucose using the standard hexokinase microtechnique and serum insulin using the radioimmunoassay technique as described by Bates and Garrison (1971, *In Laboratory Diagnosis of Endocrine Disorders*, Sunderman and Sunderman, Jr. (eds.), Green, St. Louis, Missouri, pp. 332-334). Two other squirrels which appeared normal were also fasted for 24 hr, bled and tested for D-glucose and serum insulin levels.

A complete necropsy was performed on both the diabetic and normal squirrels and multiple tissues were collected and fixed in 10% neutral buffered formalin. The tissues were fixed for 48 hr, trimmed, placed in plastic cassettes and processed on an automatic tissue processor on a 4-hr cycle. Tissues were embedded in paraffin, sectioned at 6 µm and stained with hematoxylin and eosin. Pancreatic tissue for electron microscopy was fixed in 0.1 M cacodylate-buffered 4% glutaraldehyde at pH 7.3 for 3 hr. The tissue was post-fixed in cacodylate-buffered 1% osmium tetroxide for 1 hr. The tissue was then dehydrated in a graded series of ethanol solutions, cleared in acetone and infiltrated and embedded in Epon-Araldite. Semi-thin sections (1 µm) were cut on glass

Received for publication 10 January 1984.

TABLE 1. Serum glucose and serum insulin in 24-hr fasted diabetic and non-diabetic golden-mantled ground squirrels.

Squirrel no.	Status	Serum glucose (mg/dl)	Serum insulin ( $\mu$ U/ml)
1	Diabetic	475	15
2	Non-diabetic	180	83
3	Non-diabetic	170	75

knives, stained with toluidine blue and examined by light microscopy to locate pancreatic islets. When islets were located on the semi-thin sections, ultrathin sections (100 nm) were cut, mounted on copper grids and stained with lead citrate and uranyl acetate. Sections were examined and micrographs were taken on a Philips EM201 transmission electron microscope.

Clinical observations of the diabetic ground squirrel demonstrated a 24-hr water intake of 125–220 ml ( $\bar{x}$  = 182), 24-hr urine output of 65–130 ml ( $\bar{x}$  = 103), glucose levels of 500+ mg/dl urine, protein levels of 30–300 mg albumin/dl urine, negative to moderate urine ketone levels, and a mean urine pH of 8.0. The mean 24-hr water intake was roughly 2–3 times that of normal golden-mantled ground squirrels, thus demonstrating severe polydipsia. The 24-hr urine outputs were similar to those reported by Stuhlman et al. (1977, op. cit.) for diabetic thirteen-lined ground squirrels and represent a 5–7-fold increase over normal outputs, demonstrating severe polyuria. Urinalysis results were similar to those found in diabetic thirteen-lined ground squirrels and were highly indicative of diabetes mellitus (Stuhlman et al., 1977, op. cit.).

Table 1 summarizes the serum glucose and serum insulin of both fasted diabetic and non-diabetic golden-mantled ground squirrels. The glucose levels were highly elevated and insulin levels greatly depressed in the diabetic squirrel. Both conditions indicated the presence of diabetes mellitus. Furthermore, these data fall

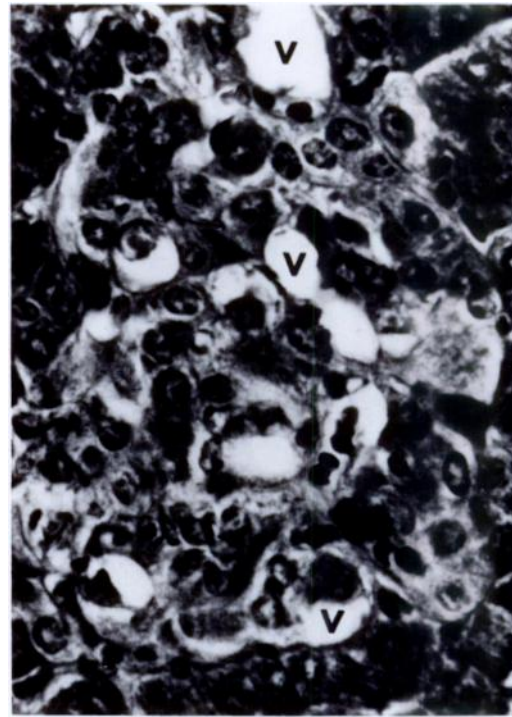


FIGURE 1. Photomicrograph of vacuolated cells (V) within a pancreatic islet of a diabetic golden-mantled ground squirrel. Hematoxylin and eosin,  $\times 600$ .

within the ranges seen in fasted thirteen-lined ground squirrels (serum glucose—422.7 vs. 189.6 and serum insulin 16.0 vs. 88.3 for diabetic and non-diabetic respectively) (Stuhlman et al., 1977, op. cit.).

Histopathologically, lesions were confined to the eye and pancreas. The lens of one eye contained a cataract. Multiple globular, well circumscribed areas were present beneath the capsular surface of the lens. The retina of the same eye with the cataract contained both normal and focal areas of atrophy. The areas were characterized by the loss of cones with subsequent reduction of the outer nuclear layer.

The islets in the pancreas were reduced markedly in number from normal animals. Multiple vacuoles were present in the cytoplasm of the affected cells and ap-

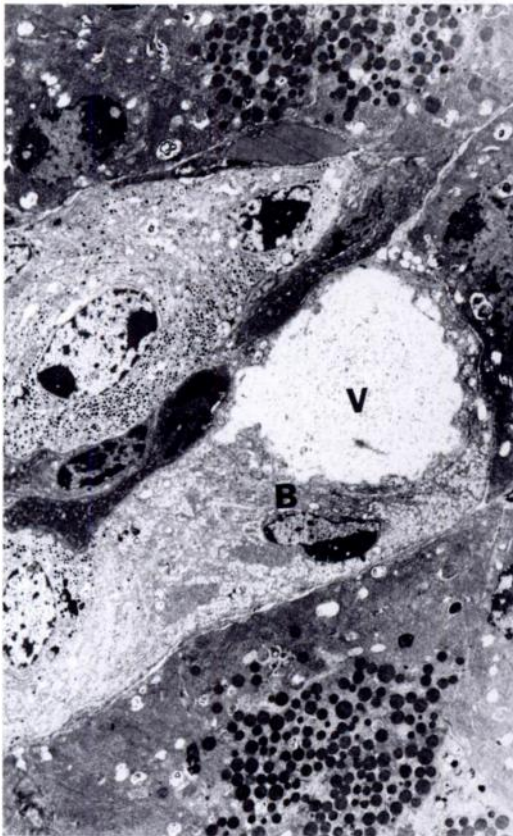


FIGURE 2. Electron micrograph showing a vacuole (V) in a  $\beta$  (B) cell of the pancreas of a diabetic golden-mantled ground squirrel. Lead citrate, uranyl acetate,  $\times 3,600$ .

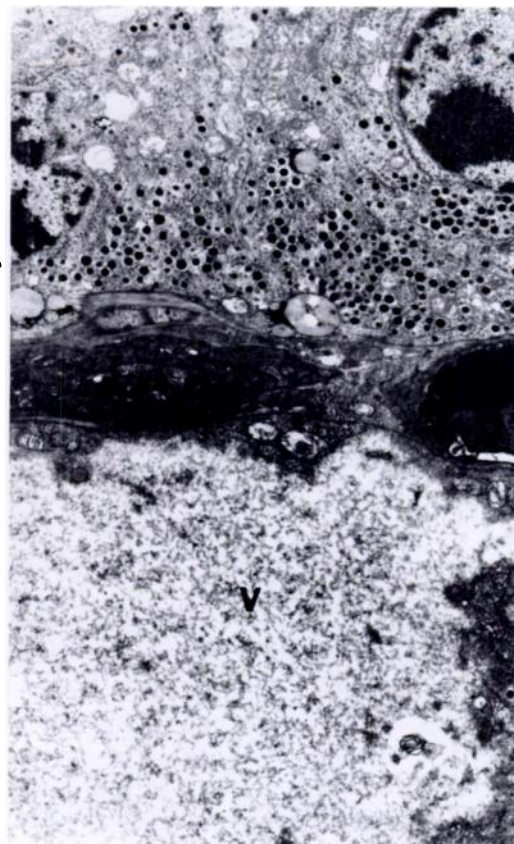


FIGURE 3. Higher magnification of the vacuole (V) shown in Figure 2, of the pancreas of a diabetic golden-mantled ground squirrel. Lead citrate, uranyl acetate,  $\times 10,800$ .

peared to be of a degenerative nature, but on light microscopy it could not be determined if the vacuoles were present in  $\alpha$  or  $\beta$  cells (Fig. 1). Figure 2 is an electron micrograph showing a portion of an islet surrounded by acinar tissue and illustrating both  $\alpha$  (those containing closely packed dark granules) and  $\beta$  cells. A  $\beta$  degenerative cell containing a large vacuole in the cytoplasm is also illustrated. Figure 3 is a higher magnification showing the cellular degeneration associated with the vacuole.

The clinical and histopathological data demonstrate clearly the presence of spontaneous diabetes mellitus in a golden-mantled ground squirrel. While sponta-

neous diabetes has been reported in a large number of mammals, this is the first report of diabetes in this species and only the second in a species of mammal which undergoes true hibernation. This is significant in that the golden-mantled ground squirrel's weight gain prior to hibernation is in the range of 80% of its fat-free weight (Jameson and Mead, 1964, *J. Mammal.* 45: 359–364). A diabetic condition during this pre-hibernation period would prove fatal undoubtedly.

This study was sponsored, in part, by the UALR College of Science Office of Research, Science and Technology.