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Influence of Spontaneous Diabetes on Tissue Status of Zinc, Copper, and Manganese in the BB Wistar Rat[1] (42182)

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Abstract. The concentrations of zinc, copper, and manganese in liver, kidney, duodenum, pancreas, testes, bone, and serum from control and untreated, spontaneously diabetic BB Wistar rats were compared. Chronic insulin deficiency resulted in significant alterations in the concentrations of one or more of these essential micronutrients in several tissues. The amounts of zinc and copper bound to metallothionein in the liver and kidney of untreated spontaneously diabetic rats were also markedly increased. The tissue trace metal status in diabetic rats was altered similarly in both male and female rats. Daily injections of insulin blocked many of the changes in the tissue concentrations of the metals. The effects of spontaneous diabetes on tissue trace metal status are quite similar to those reported for chemically induced diabetes. Thus, these results demonstrate that chronic endocrine imbalance is responsible for a series of tissue specific changes in the transport and metabolism of zinc, copper, and manganese.

Recent reports from several laboratories have shown that chemical induction of the insulin-deficient, diabetic condition in the laboratory rat is associated with marked alterations in the metabolism of essential trace metals. More specifically, the absorption of zinc and copper (1), zinc retention (2), the concentrations and metabolism of zinc (3–7), copper (3, 5, 8), iron (4, 6), and manganese (3, 7, 9) in some tissues, and the urinary excretion of zinc, copper, and iron (10) are all increased in untreated, streptozotocin-diabetic rats. The degree of the alterations is influenced by the severity and duration of the diabetic condition (5) and dietary factors (6), but is independent of the strain and sex of the rat (11). Failla and Kiser (5) have proposed that the observed changes in trace metal metabolism are due to the endocrine imbalance associated with the diabetic condition. However, definitive evidence to exclude the possibility that the alterations result from cytotoxic effects of the diabetogenic drug, streptozotocin, on extrapancreatic tissues is lacking. Such information is imperative, since streptozotocin may damage kidney tubules and the liver (12, 13). These are two of the primary tissues in which the metabolism of trace metals is altered by chemical diabetes.

The purpose of the present study was to examine the trace metal status of tissues from untreated and insulin treated, spontaneously diabetic BB Wistar rats. The onset of diabetes in this animal is sudden and associated with insulitis, destruction of the pancreatic β cells, and the development of hyperglycemic ketoacidosis. Various characteristics of this animal model have been reviewed (14–16). The results support our hypothesis that the endocrine imbalance of the untreated diabetic state results in marked changes in the concentrations of zinc, copper, and manganese in selected tissues.

Materials and Methods. *Animals.* Details of animal housing and care have been previously reported (17). A total of 23 BB Wistar spontaneously diabetic rats and 17 BB Wistar nondiabetic rats of similar age were examined. Similar numbers of male and female rats were included in each group. The mean age of onset of the diabetic condition was 106 ± 5 days (range of 79–147 days). All diabetic rats were weighed and the level of glucose in the urine was estimated each day (Testape, Eli Lilly). Eleven diabetic rats were randomly selected to receive daily injections (subcutaneous) of protamine zinc (U-40) insulin (Connaught Laboratory, Toronto). The dose of insulin administered to each diabetic animal (2–4 units)

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prevented ketonuria and resulted in a growth rate (increase in body weight) similar to that of nondiabetic animals.

Untreated diabetic rats were sacrificed at either 14 days or 25–28 days after diabetes was detected. Equivalent numbers of insulin-treated animals with diabetes of similar duration and nondiabetic rats of similar age were also sacrificed at these times. Plasma glucose levels at the time of sacrifice were 142 ± 5, 628 ± 31, and 358 ± 45 for nondiabetic, untreated diabetic, and insulin-treated diabetic rats, respectively.

Tissue analysis. The following tissues were collected from each animal: liver, kidney, duodenum (20-cm segment from the pyloric sphincter), femur, testes (when applicable), and blood. Residual blood and luminal contents were flushed from the liver and duodenum, respectively, with ice-cold saline. Tissues were stored in polyethylene bags at −20°C until analysis. Serum was prepared from blood that had been collected by posterior aorta puncture.

Tissues, except serum, were dried at 105°C for 20 hr, weighed, and then ashed at 450°C for 24 hr. Ash was dissolved in 0.12 *N* HCl ("Ultrex", J. T. Baker) and diluted appropriately for the analysis of zinc, copper, and manganese concentrations by atomic absorption spectrophotometry (Perkin–Elmer Model 560) with an air:acetylene flame. Identical handling and analysis of bovine liver 1577 from the National Bureau of Standards demonstrated that the recovery of all three metals in samples exceeded 95%. Serum samples were diluted 1:4 with trichloroacetic acid (final concentration 5%) and the acid soluble fraction was analyzed for zinc, copper, and iron.

Metallothionein. The concentrations of zinc and copper associated with metallothionein in the cytosol fraction of livers and kidneys were determined by gel filtration chromatography as reported previously (5). Briefly, similar amounts of tissue from the animals in each group were pooled and homogenized in buffer containing 250 m*M* sucrose, 20 m*M* Tris–acetate, 1 m*M* 2-mercaptoethanol, pH 8.6. The soluble fraction (cytosol) was prepared by centrifugation of the homogenate at 105,000*g* for 90 min at 4°C. Aliquots (4 ml) of cytosol were applied to columns (2.5 × 60 cm) containing borohydride-treated Sephadex G-75 that was equilibrated with buffer (20 m*M* Tris–acetate, 1 m*M* 2-mercaptoethanol, pH 8.6). Cytosol species were eluted at a rate of 20–25 ml/hr and 4.0-ml fractions were collected. The elution characteristics (V_v, V_{mt}, and V_t) of the columns were defined by examining the separation of a solution containing Blue Dextran, purified rat liver zinc-metallothionein, and potassium dichromate. The concentrations of zinc and copper in each fraction were directly measured by atomic absorption spectrophotometry. Recovery of zinc and copper from the columns exceeded 90%.

The levels of zinc and copper bound to metallothionein were determined by summing the amount of each metal in fractions that eluted at 1.8–2.1 times the void volume (V_v). Samples were compared by expressing the relative amounts of metallothionein as microgram metal bound to metallothionein per gram cytosol protein. The amount of protein applied to each column was measured by the microbiuret procedure (18).

Analysis of data. Results are given as means ± SE. Data were subjected to analysis of variance and Duncan's multiple range test to determine significant differences at $\alpha < 0.05$ (19).

Results. *Tissue concentrations of trace metals.* There were no significant differences between the trace metal concentrations of male and female rats in each group. Therefore, results for both sexes have been combined for presentation below.

Zinc concentrations in the liver, kidney, and pancreas of untreated, spontaneously diabetic rats were elevated approximately 20% (Table I). In contrast, insulin deficiency did not affect the concentration of zinc in intestine, testes, and bone. Daily treatment of diabetic rats with insulin reduced the concentration of zinc in liver and kidney, but not pancreas, to control levels. The concentration of zinc was slightly, but significantly, increased in the small intestine of insulin-treated diabetic rats.

Hepatic and renal concentrations of copper were 14 and 275% higher, respectively, in untreated diabetic rats than in control animals (Table I). Insulin-treated diabetic and control rats had similar concentrations of copper in the liver, but the level of this metal in the kidney of spontaneously diabetic rats receiving insulin was 48% higher than in controls. Neither the diabetic condition itself nor insulin

TABLE I. INFLUENCE OF THE INSULIN DEFICIENCY ON THE CONCENTRATIONS OF ZINC, COPPER, AND MANGANESE IN VARIOUS TISSUES OF BB/W RAT

Tissue	Metal	Metal concentration (μg/g dry wt)[d] Control (N = 17)	Diabetic (N = 12)	Insulin-treated diabetic (N = 11)
Liver	Zn	99.6 ± 3.0[a]	113.9 ± 2.3[b]	102.4 ± 3.1[a]
	Cu	14.0 ± 0.7[a]	15.9 ± 0.5[b]	14.6 ± 0.6[a]
	Mn	8.6 ± 0.6[a]	16.0 ± 1.1[b]	9.8 ± 0.6[a]
Kidney	Zn	93.4 ± 1.9[a]	114.2 ± 2.7[b]	98.7 ± 2.1[a]
	Cu	29.3 ± 5.0[a]	80.7 ± 7.1[b]	43.3 ± 4.5[c]
	Mn	4.0 ± 0.1[a]	4.7 ± 0.3[c]	3.8 ± 0.2[a]
Intestine	Zn	90.7 ± 2.0[a]	94.4 ± 2.9[a,b]	98.3 ± 1.9[b]
	Cu	8.2 ± 0.5[a,b]	7.3 ± 0.6[a]	9.4 ± 0.4[b]
	Mn	5.0 ± 0.5[a]	5.7 ± 0.5[a]	5.4 ± 0.4[a]
Pancreas	Zn	66.2 ± 3.4[a]	80.2 ± 4.5[b]	76.8 ± 4.9[a,b]
	Cu	7.4 ± 0.4	6.8 ± 0.4	7.3 ± 0.5
	Mn	3.6 ± 0.3[a]	6.3 ± 0.4[b]	4.2 ± 0.4[a]
Testes	Zn	183.8 ± 4.5	177.7 ± 3.1[a]	178.3 ± 4.6[b]
	Cu	15.8 ± 0.4	15.7 ± 0.6	16.0 ± 0.2
	Mn	2.4 ± 0.1[a]	2.7 ± 0.1[b]	2.3 ± 0.1[a]
Bone	Zn	231.4 ± 5.1	238.4 ± 4.8	229.7 ± 5.4

[a-c] Values in a row with different letters as superscripts significantly differ at $\alpha < 0.05$.
[d] Means ± SE.

treatment influenced copper concentrations in pancreas and testes. However, diabetic rats treated with insulin had higher concentrations of copper in intestine than control and untreated diabetic rats.

Manganese concentrations in untreated diabetic liver, kidney, pancreas, and testes were elevated by 86, 18, 75, and 13%, respectively, in comparison with the concentrations present in the same tissues from control animals (Table I). Tissues from insulin-treated diabetic rats and control animals had similar concentrations of manganese.

Untreated diabetic rats were also characterized by hyperzincemia, hypocupremia, and hypomagnesemia (Table II). Insulin treatment elevated serum copper levels, but did not significantly lower serum zinc. Plasma concentrations of iron and calcium (Table II) and bone calcium (data not shown) were similar in all groups.

In those tissues of the untreated diabetic rat in which the concentration of one or more of the trace metals was significantly different from control and insulin-treated diabetic rats (Tables I and II), the influence of the duration of the diabetic condition on the degree of change in trace metal concentrations varied. Significantly higher concentrations (μg/g dry wt) of the indicated metals were present in the following tissues at 25–28 days after detection of insulin deficiency compared to 14 days: liver

TABLE II. CONCENTRATIONS OF ESSENTIAL METALS IN PLASMA FROM CONTROL, SPONTANEOUSLY DIABETIC, AND INSULIN-TREATED DIABETIC BB/W RATS

Element	Plasma metal concentration (μg/ml)[d] Control (N = 17)	Diabetic (N = 12)	Insulin-treated (N = 11)
Zn	1.36 ± 0.04[a]	1.69 ± 0.10[b]	1.63 ± 0.08[b]
Cu	1.20 ± 0.05[a]	1.04 ± 0.05[b]	1.36 ± 0.08[c]
Fe	2.74 ± 0.20	2.68 ± 0.14	2.87 ± 0.17
Ca	102.5 ± 2.1	107.8 ± 2.6	106.4 ± 1.3
Mg	17.6 ± 0.3[a]	16.1 ± 0.6[b]	16.7 ± 0.6[a,b]

[a-c] Values in a row with different letters as superscripts differ at $\alpha < 0.05$.
[d] Means ± SE.

zinc (119 ± 4 vs 109 ± 4) and manganese (18 ± 1 vs 15 ± 1), kidney copper (98 ± 13 vs 69 ± 3), and pancreas zinc (87 ± 4 vs 76 ± 4). In contrast, the concentrations of zinc in kidney and plasma, copper in liver and plasma, and manganese in kidney and pancreas from untreated spontaneously diabetic rats were elevated to a similar extent over controls at 14 and 25–28 days after onset of diabetes. The concentration of copper in kidney of insulin-treated diabetic rats at 14 and 25–28 days after detection of the insulin-deficient state was also similar, viz., 40 ± 4 and 46 ± 5 μg/g dry wt, respectively, and significantly higher than normal.

Hepatic and renal metallothionein. The molecular distribution of zinc and copper in cytosol prepared from the livers and kidneys of control, untreated diabetic, and insulin-treated diabetic rats was compared by Sephadex G-75 gel filtration chromatography. All of the additional complement of zinc in liver and kidney and copper in liver of untreated diabetic rats was present in the cytosol fraction. In contrast, the cytosol and particulate (105,000*g* pellet) fractions each contained approximately one-half of the additional load of copper present in the kidney of untreated diabetic rats.

Zinc in cytosol from liver and kidney of all groups eluted in three distinct peaks, viz., at void volume, at 1.4–1.6 times void volume, and with metallothionein (1.8–2.1 times void volume). The quantity of zinc associated with metallothionein in the liver and kidney of untreated diabetic rats was 3.5 and 2.0 times higher, respectively, than in controls (Table III). Insulin-treated rats and controls had similar levels of zinc bound to hepatic metallothionein. Cytosol from untreated and insulin-treated diabetic rats also had markedly less zinc eluting at 1.4–1.6 times void volume than controls (data not shown).

Copper in cytosol prepared from the livers and kidneys of all groups eluted in two peaks, viz., at 1.4–1.6 times void volume (presumably superoxide dismutase) and with metallothionein. Cytosol from the liver and kidney of untreated diabetic rats had 2.6 and 2.8 times, respectively, as much copper bound to metallothionein as control samples (Table III). The concentration of copper associated with metallothionein was similar in livers from control and insulin-treated diabetic rats. However, there was 56% more copper bound to metallothionein in renal cytosol from insulin-treated animals than control animals. Hepatic and renal cytosol from all groups had similar amounts of copper eluting at 1.4–1.6 times void volume, suggesting that the diabetic condition did not alter tissue levels of superoxide dismutase.

TABLE III. INFLUENCE OF INSULIN STATUS ON THE CONCENTRATIONS OF ZINC AND COPPER BOUND TO METALLOTHIONEIN (MT) IN CYTOSOL FROM POOLED SAMPLES OF LIVERS AND KIDNEYS OF BB/W RATS

		μg metal bound to MT/g cytosol protein		
Tissue	Metal	Control	Diabetic	Insulin-treated diabetic
Liver	Zn	17.4	60.9	23.6
	Cu	3.4	8.8	1.4
Kidney	Zn	48.7	96.0	71.2
	Cu	61.0	170.6	93.4

Discussion. The results from the present study concerning the influence of the untreated diabetic condition on tissue concentrations of trace metals in the spontaneously diabetic BB Wistar rat are generally consistent with earlier investigations with streptozotocin-diabetic rats. Both spontaneously diabetic and chemically diabetic rats had significantly higher concentrations of zinc, copper, and manganese in liver [Table I; Refs. (3, 5–7, 9, 11)], zinc and copper in kidney [Table I; (3, 5, 6, 8, 11)], and zinc in plasma [Table 2; (3)] than their respective nondiabetic controls. These changes occurred to the same extent in both male and female diabetic rats [this study; (11)]. Increased levels of zinc and copper were also bound to metallothionein in cytosol prepared from the livers and kidneys of spontaneously diabetic (Table III) and streptozotocin-diabetic rats (3, 5, 9, 11). Despite the presence of elevated levels of zinc and copper in the liver and kidney of spontaneously diabetic rats, the concentrations of these metals in duodenum were not affected by chronic insulin deficiency (Table I). This observation is similar to the findings of Failla and associates (1, 3) and Ghishan and Greene (2) in the streptozotocin-diabetic rats. In contrast, Johnson and Evans (6) reported that duodenal zinc

and copper concentrations were significantly higher in streptozotocin-diabetic rats than in nondiabetic controls. The basis for this discrepancy is unknown. Plasma levels of calcium and iron in untreated spontaneously diabetic rats did not differ from that of nondiabetic controls, while slight hypomagnesemia was detected in the former (Table II). Chronic insulin deficiency did not alter plasma levels of calcium, magnesium, and iron in streptozotocin-diabetic rats (20, 21).

Daily administration of insulin to streptozotocin-diabetic rats usually maintained the concentrations of trace metals in tissues at control levels (3, 10). However, renal copper concentrations in severely diabetic rats treated with insulin were significantly higher than in nondiabetic control animals (10). Since streptozotocin may be nephrotoxic (12, 13), we were concerned that the failure of treatment with insulin to maintain normal copper status in the kidney might be due to drug-mediated cellular damage. In the present study, renal concentrations of copper (Table I) and copper-metallothionein (Table III) were 48 and 46% higher, respectively, in insulin-treated, spontaneously diabetic rats than in control rats. The concentrations of zinc, copper, and manganese in extrarenal tissues of insulin-treated, spontaneously diabetic rats were generally similar to those of nondiabetic controls (Tables I and II).

Several differences between spontaneously diabetic and chemically diabetic rats were also noted for tissue concentrations of trace metals. First, the renal concentration of manganese was significantly elevated in untreated spontaneously diabetic rats (Table I), but not in untreated streptozotocin diabetic rats (3, 9). Elevated concentrations of manganese have been reported in the kidneys of streptozotocin-diabetic mice (11). Second, Johnson and Evans (6) reported a two to fourfold higher concentration of copper, but no alteration in the concentration of zinc, in the pancreas of streptozotocin-diabetic rats. In contrast, pancreatic zinc levels were elevated by 20% and pancreatic copper concentrations were not altered in spontaneously diabetic rats compared to the nondiabetic animals (Table I). Third, spontaneous diabetes did not affect zinc concentrations in bone (Table I). Rosholt and Hegarty (4) found significantly higher concentrations of zinc in femur, humerus, and scapula from chronic (79 day), untreated streptozotocin-diabetic rats than in bones from nondiabetic rats. The conflicting data from these two studies are probably due to differences in the duration of the insulin-deficient condition. Zinc concentrations in bones from rats with streptozotocin-induced diabetes of 2- to 3-weeks duration were similar to control levels (3, 6). Finally, the decreased concentration of copper in the plasma of spontaneously diabetic rats (Table I) was not observed in streptozotocin-diabetic rats (3).

Untreated streptozotocin-diabetic rats approximately double daily caloric intake by 1 week after induction of the insulin-deficient condition. Previous studies have shown that the elevated concentrations of one or more trace metals in specific tissues of the streptozotocin-diabetic rat were also significantly elevated when the quantities of these elements ingested per day were similar to those of normal rats (3, 6). Thus, the increased tissue concentrations of the trace metals were not attributable to hyperphagia alone. Rather, the altered endocrine status of the streptozotocin-diabetic rat affects the absorption (1, 2), tissue distribution and metabolism (3, 9, 21, 22), and retention (9, 22) of both endogenous and dietary trace metals. Although we did not monitor food intake in the present study, others have reported that moderately ketotic and stable spontaneously diabetic BB Wistar rats increase food consumption by approximately 25% (23). It is unlikely that this slight increase in the intake of trace metals accounted for the marked accumulation of these metals in some tissues. Another similarity between chemically diabetic and spontaneously diabetic rats was the influence of the duration of insulin deficiency on tissue trace metal status. Most striking in both animal models is the preferential and continual accumulation of copper in the kidney [Table I; Refs. (5, 8)]. In most other tissues of the spontaneous-diabetic rat in which the concentrations of trace metals were altered by the diabetic state, the changes observed after 2 weeks were similar in magnitude to those after 4 weeks. We have proposed that the early changes in tissue trace metal levels in response to the onset of the diabetic condition reflect those alterations required for metabolic adaptation to insulin deficiency (5).

housed in the MUSC animal facilities and sacrificed at the ages indicated for each experiment. The number of mice used for each experiment is shown in Figs. 1 and 3. The number of Tsk mice used always equaled the number of +/+ mice used in each experiment. At the desired age, mice were anesthesized with 0.2 ml Avertin followed by cervical dislocation. A sample of shaved skin was taken from the dorsal surface between the shoulder blades with a 6-mm biopsy punch. Samples were fixed and processed for light microscopy. Five micrometer sections were cut; hematoxylin and eosin and Giemsa stains were performed. Sections were projected onto graph paper, outlines drawn, and the paper cut out and weighed to two decimal places. A transparent measure was projected similarly to obtain a curve of standard areas from which the total area in square millimeters of each section was determined. Mast cells in the entire section were counted and the following equation was used to determine the number of mast cells per mm square millimeter:

$$\text{mast cells/mm}^2 = \frac{\text{total mast cell number}}{\text{total section area}}.$$

Mast cells were also classified as to the degree of degranulation from 0 to 4+ as follows:

0 = no extracellular granules visible
1+ = cell and nucleus clearly distinguishable with 2–10 extracellular granules visible
2+ = cell and nucleus clearly distinguishable with 11–25 extracellular granules visible
3+ = cell and nucleus barely visible through extracellular granules numbering 26–50
4+ = cell and/or nucleus difficult to find; massive number of granules (>50) clustered in an area two to five times the size of the cell, and many times accompanied by a diffuse interstitial distribution of granules.

The cylindrical skin biopsy was cut in half along its long axis perpendicular to the epidermis. The tissue was placed in the embedding block with the flat surface down, and cut parallel to the flat surface with epidermis up. The measurement of the width of the subcutaneous fibrous layer was performed on a Videoplan image analysis system coupled to a photomicroscope (Zeiss, West Germany). The actual measurements were performed interactively on a digitizing pad using the Videoplan's y-x software.

Except for the recognized difference in hair color (Tsk mice have black coats; +/+, beige) and skin thickness, no differences were observed in growth, behavior, appetite, or age-corrected weights between Tsk and +/+ mice.

Results. In both Tsk and +/+ mice, as has been shown in other species, the total number of mast cells decreases with age. In the Tsk mouse, the number of mast cells and/or the degree of degranulation differ from +/+ mice at comparable ages. Figure 1 shows the num-

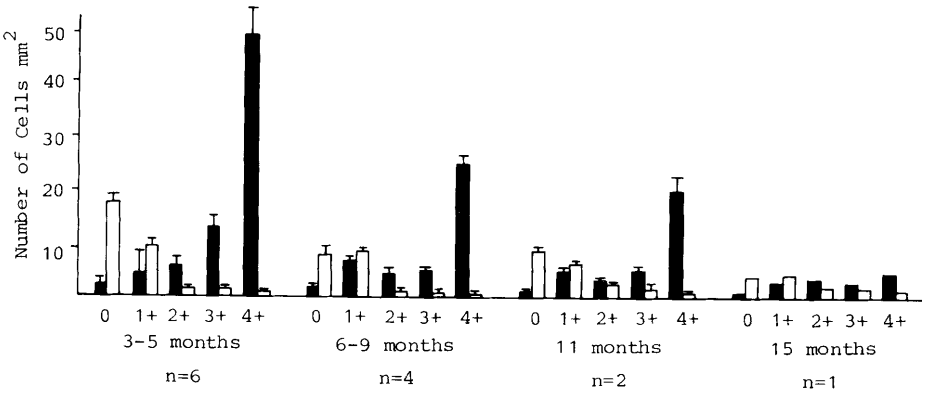

FIG. 1. Number of mast cells/mm^2 skin in each degranulation state 0 to 4+ for Tsk (closed bars) and +/+ (open bars) mice. *n* = number of sections quantified and number of mice studied. Brackets represent one standard deviation.

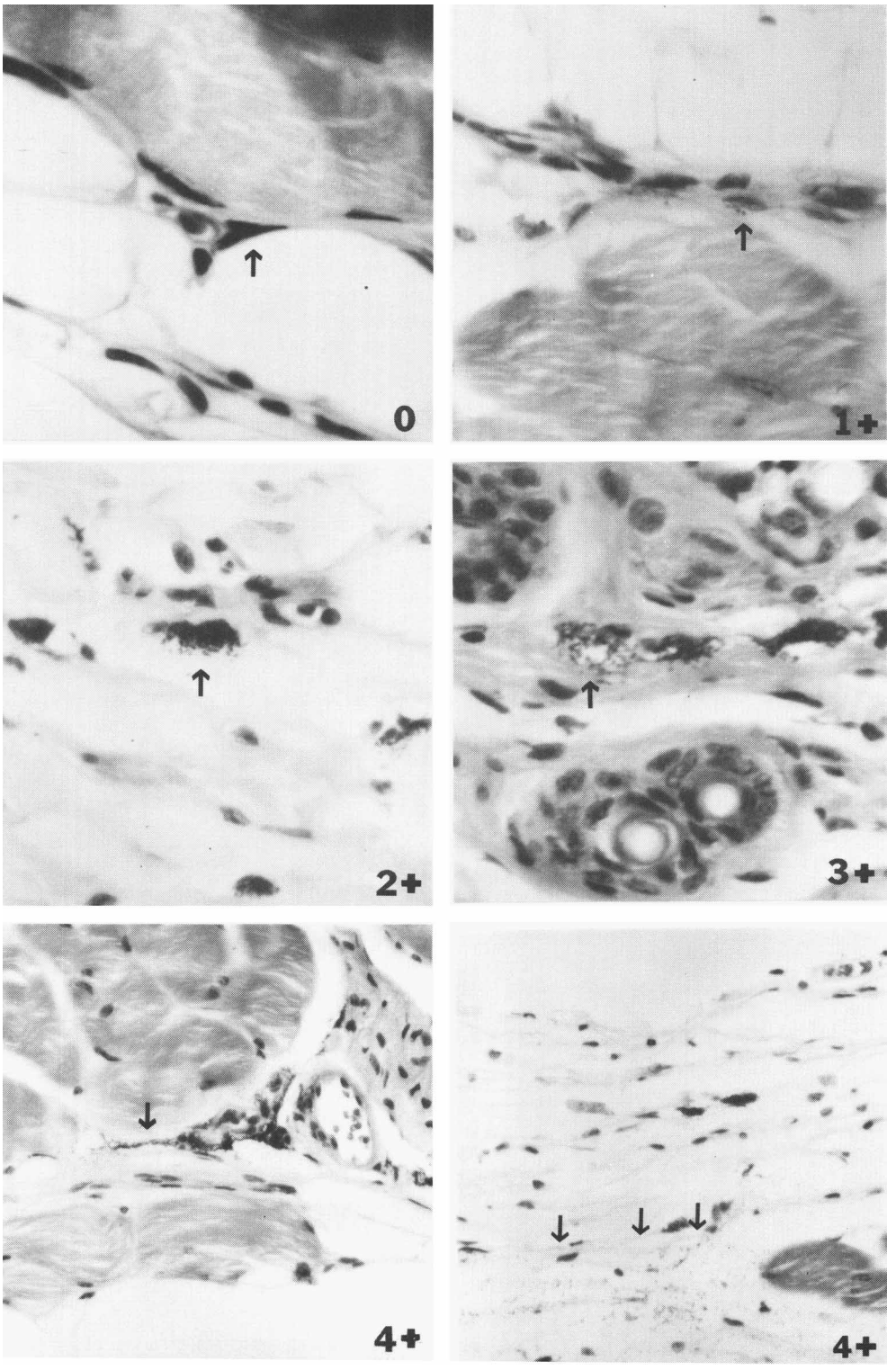

FIG. 2. Photomicrographs of mast cells in each of the degranulation states (0 to 4+) (400×). The 0 state shows no granules outside the cell. The 1+ cell has 2 extracellular granules. There are approximately 20 granules outside the 2+ cell. The 3+ cell has approximately 40 extracellular granules and the cell looks broken up compared to the 0 state cell. The 4+ cell (left picture) shows many granules in a large area and the cell is difficult to see. The 4+ area (right picture) is a large accumulation of granules. The cell(s) is not visible as a distinct entity.

ber of cells per square millimeter in Tsk and +/+ for each degranulation category (0–4+) at ages between 3 and 15 months. At 3, 5 and 6 months, mast cell numbers in Tsk skin are more than twofold greater than in +/+ skin. At 11 months, the proportion is 1.8, and at 15 months, the number of skin mast cells is similar in Tsk and +/+ and significantly reduced from the number in younger mice. That younger Tsk mice have more mast cells is consistent with a pathogenetic role for these cells in the fibrotic process.

Not only do the number of cells in the affected and normal mice differ, but also the degree of degranulation differs. At 5 months, the Tsk mouse has few cells exhibiting no visible degranulation with the majority exhibiting 4+ degranulation. At 5 months, most mast cells in the +/+ mouse are normal (0) and very few are degranulated (4+ state). At 15 months, even though the total mast cell number per square millimeter is nearly equivalent in Tsk and +/+, the proportion of highly degranulated cells in Tsk remains increased. Figure 2 shows representative examples of degranulation classified from 0 to 4+.

Figure 3 compares the widths of the fibrous layer at various ages. Even at young ages, there is more fibrous tissue in the Tsk than in the +/+ mouse; between 6 and 9 months a large increase in the width of the layer occurs in the Tsk mouse with little to no change in the +/+ skin.

Other histopathologic abnormalities of the Tsk mouse are being prepared for separate publication. Mast cell abnormalities have only been observed in the skin.

Discussion. We have demonstrated in Tsk mouse skin an absolute increase in the width of a subcutaneous fibrous layer, in the number of mast cells, and in the degree of mast cell degranulation, all compared to +/+ skin.

An increase in the density of dermal mast cells has been noted in various skin diseases, including urticaria pigmentosa, basal cell carcinoma, neurofibromatosis and lichenified atopic eczema; therefore, it seems reasonable that the mast cell density could be increased in other skin disorders as in the Tsk mouse and scleroderma in man.

Increased numbers of mast cells in scleroderma skin have been reported by Hawkins *et al.* (7). They noted that mast cell counts were greater in involved skin than uninvolved skin or in control skin; unusual degrees of mast cell degranulation were not seen. Skin lesions resembling those of scleroderma have been observed in chronic graft-versus-host disease (8–11); mast-cell dysfunction has been noted in a mouse model of chronic GVHD (12, 13).

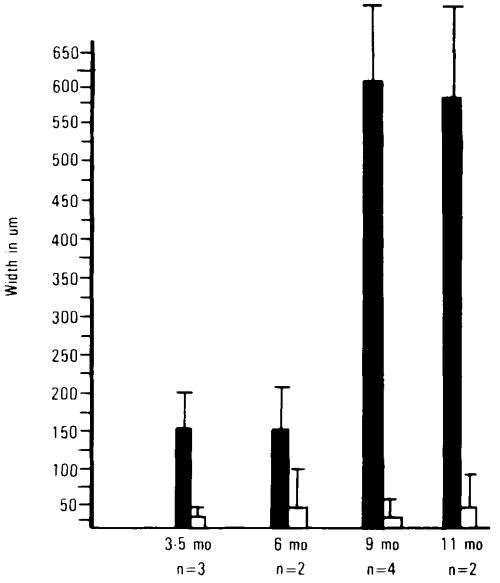

FIG. 3. Width of subcutaneous fibrous layer in Tsk (closed bars) and +/+ (open bars) skin. *n* = number of sections quantified. Brackets represent one standard deviation.

Greenberg and Burnstock have described a cell-to-cell interaction between mast cells and both fibroblasts and endothelial cells (14). Mast cells could affect the behavior of fibroblasts and be important in the pathogenesis of fibrosis in the Tsk mouse. The fact that these cells are present in abundance and exhibit massive degranulation at early age when the subcutaneous fibrous layer has not yet reached maximum width suggests an association between mast cells and fibrosis in the Tsk mouse. Experiments are underway to inhibit mast cell function followed by histological observation of the appearance of fibrosis.

There is evidence that mucosal mast cells may be under T-cell control, and that, in turn, they may modulate T cells and other immune cells (15–20). The mechanisms of regulation

of connective tissue mast cells are unknown. Mast cell differentiation appears to be regulated by factors from $Ly1^+2^-Ia^-$ T cells (20). Also, similarities have been found between mast cells and both natural killer and suppressor T cells (21–23). Furthermore, there is evidence that mast cell degranulation, triggered by compound 48/80 or Polymixin B, stimulates fibroblast proliferation and collagen deposition (24, 25). When immune responding animal strains are shown to have plentiful mast cells, it is difficult to determine whether mast cells lead to immunoresponsiveness or vice versa. In the Tsk mouse, fibrosis could be mediated directly by mast cell products or indirectly through IgE or cell-mediated immune responses which recruit mast cells. Further study is needed to dissect these interacting influences in the Tsk mouse.

In conclusion, we have observed an increased number of mast cells in the skin of Tsk mice with most of the cells exhibiting increased degranulation, accompanied by an increase in fibrosis with age, raising the possibility that mast cells may play a role in the initiation of fibrosis in the skin of Tsk mice. Thus, the Tsk mouse may be a suitable setting to explore the longstanding and well-studied interactions between mast cells, wound healing, and fibrosis.

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1. Stites DP, Stobo JO, Fudenberg HH, Wells JV. Basic and Clinical Immunology. Calif., Lange Medical Publications, p87, 1982.
2. Wasserman SI. The mast cell and the inflammatory response. In: Pepys J, Edwards AM, eds. The Mast Cell. Its Role in Health and Disease. London, Pitman, pp9–20, 1979.
3. Friedhoff LT, Seibold JR, Kim HC, Simester KS. Serotonin-induced platelet aggregation in systemic scleroderma. Clin Exp Rheumatol **2**:119–123, 1984.
4. Green MC, Sweet HO, Bunker LE. Tight skin, a new mutation of the mouse causing excessive growth of connective tissue and skeleton. Amer J Pathol **82**:493–512, 1976.
5. Menton DN, Hess RA. The ultrastructure of collagen in the dermis of tight-skin (Tsk) mutant mice. J Invest Dermatol **74**:139–147, 1980.
6. Ross SC, Osborn TG, Dorner RW, Zuckner J. Glycosaminoglycan content in the skin of the tight-skin mouse. Arthritis Rheum **26**:653–657, 1983.
7. Hawkins RA, Claman HN, Clark RAF, Steigerwald JC. Increased dermal mast cell populations in progressive systemic sclerosis: A link in chronic fibrosis? Ann Intern Med**102**:182–186, 1985.
8. Furst DE, Clements PJ, Graze P, Gale R, Roberts N. A syndrome resembling progressive systemic sclerosis after bone marrow transplantation: A model for scleroderma? Arthritis Rheum **22**:904–910, 1979.
9. Lawly TJ, Peck GL, Moutsopoulos HM, Gratwohl AA, Deisseroth AB. Scleroderma, Sjogren-like syndrome, and chronic graft-versus-host disease. Ann Intern Med **87**:707–709, 1977.
10. Jaffee BD, Claman HN. Chronic graft-versus-host disease (GVHD) as a model for scleroderma. 1. Descriptions of model systems. Cell Immunol **77**:1–12, 1983.
11. Herzog P, Clements PJ, Roberts NK, Furst DE, Johnson CE, Feig SA. Case report: Progressive systemic sclerosis-like syndrome after bone marrow transplantation. Clinical, immunologic, and pathological findings. J Rheumatol **7**:56–64, 1980.
12. Charley MR, Bangert JL, Hamilton BL, Gilliam JN, Sontheimer RD. Murine graft-versus-host skin disease: A chronologic and quantitative analysis of two histologic patterns. J Invest Dermatol **81**:412–417, 1983.
13. Claman HN. Mast cell depletion in murine chronic graft-versus-host disease. J Invest Dermatol **84**:246–248, 1985.
14. Greenberg G, Burnstock G. A novel cell-to-cell interaction between mast cells and other cell types. Exp Cell Res **147**:1–13, 1983.
15. Askenase PW. The role of basophils, mast cells and vasoamines in hypersensitivity reactions with a delayed time course. Prog Allergy **23**:199–320, 1977.
16. Askenase PW. Immunopathology of parasitic diseases: Involvement of basophils and mast cells. Semin Immunopathol **2**:417–442, 1980.
17. Galli SJ, Dvorak HF. Basophils and mast cells. Structure, function, and role in hypersensitivity. In: Gupta and Good, eds. Cellular, Molecular, and Clinical Aspects of Allergic Diseases. New York, Plenum, pp1–53, 1979.
18. Plaut M. Histamine, H-1 and H-2 antihistamines and immediate hypersensitivity reactions. J Allergy Clin Immunol **63**:371–375, 1979.
19. Burnet FM. The probable relationship of some or all mast cells to the T-cell system. Cell Immunol **30**:358–360, 1977.
20. Ginsburg H, Olson EC, Huff TF, Okudaira H, Ishizaka

T. Enhancement of mast cell differentiation in vitro by T-cell factor(s). Int Arch Allergy Appl Immunol **66**:447–458, 1981.

21. Galli SJ, Dvorak AM, Dvorak HF. Basophils and mast cells: Morphologic insights into their biology, secretory patterns, and function. Prog Allergy **34**:1–141, 1984.
22. Dvorak AM, Galli SJ, Marcum JA, Nabel G, Der Simonian H, Goldin J, Monahan RA, Pyne K, Cantor H, Rosenberg RD, Dvorak HF. Cloned mouse cells with natural killer function and cloned suppressor T cells express ultrastructural and biochemical features not shared by cloned inducer T cells. J Exp Med **157**: 843–861, 1983.
23. Galli SJ, Dvorak AM, Ishizaka T, Nabel G, Der Simonian A, Cantor H, Dvorak HF. A cloned cell with natural killer function resembles basophils by ultrastructure and expresses IgE receptors. Nature (London) **298**:288–290, 1982.
24. Franzen L. Further studies on the relationship between drug-induced mast cell secretion and local cell proliferation. Acta Pathol Microbiol Scand **89**:57–62, 1981.
25. Dabrowski R, Szczepanowska A. Alterations in histamine and collagen induced in chick embryos by aminoquanidine and 48/80. Agents Actions **14**:458–460, 1984.

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