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Effect of Glucosamine Administration in Normal Rats in Comparison with Streptozotocin Treatment (37799)

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Results of previous studies have suggested that the net synthesis of hexosamine is increased in diabetic rats (1). It has been recognized also that glucosamine is readily incorporated into glycoproteins (2) and these have been implicated in the development of diabetic angiopathy. This prompted us to investigate the effects of the long-term administration of D-glucosamine on glucose metabolism and on the structure of the retinal capillaries in rats.

This paper shows that small daily doses of D-glucosamine, cause a slight impairment of glucose tolerance associated with a diminished insulinogenic response to glucose. In addition, we found a relative loss of mural cells from the retinal capillaries, consistent with changes described in the Chinese hamster (3).

Materials and Methods. Male Sprague–Dawley rats weighing between 150 and 200 g, maintained on Oriental laboratory chow and water ad lib., received a daily injection of glucosamine (250 mg/kg body wt ip) for 2 mo. Controls were injected with normal saline for the same period of time. The production of streptozotocin diabetes and the measurement of urinary and tissue hexosamine content are described in the companion paper (1).

Oral glucose tolerance tests were performed, after an overnight fast, by administering glucose (3 g/kg) via gastric tube. Blood was drawn from the tail vein, under pentobarbital anesthesia. Serum glucose was measured by the *O*-toluidine method (4); serum insulin by a double antibody method, using antibovine insulin serum (5).

Liver Enzyme Assay. After an overnight fast, livers were obtained from glucosamine treated rats, from diabetic rats maintained for 2 mo after streptozotocin injection, and from age-matched control rats. Liver homogenates were prepared in 2 vol of 0.1 *M* Tris buffer, pH 7.4 and centrifuged at 80,000g for 1 hr. The supernatant fluids were used for the determination of glucokinase (EC 2.7.1.2) activity as described by Vinuela *et al.* (6), using a Hitachi automatic spectrophotometer at 25°. Glucose 6-phosphate phosphohydrolase (G6P-ase EC 3.1.3.9) activity was assayed as described by Nordie (7). Inorganic phosphate was measured according to Fiske and SubbaRow (8).

Urinary Protein. Twenty-four hour urine specimens were collected, concentrated, and dialyzed as described previously (1). Protein was determined by the Folin–Ciocalteu method (9).

Histological Studies. Pancreatic tissue was fixed in Bouin's solution and paraffin-embedded sections were cut at 6 μm and stained with aldehyde fuchsin. Liver and kidney specimens were fixed in 10% formalin solution and paraffin-embedded sections were cut at 6 μm and stained with hematoxylin–eosin or PAS.

Retinal Preparations. Both eyes were enucleated and placed in 5% formalin solution. After at least 2 days, they were cut open and a trypsin-digested flat retinal preparation was made according to the technique of Kuwabara and stained with PAS and hematoxylin (10).

Four separate pictures of each retina were taken at × 100 magnification: two at the posterior pole and two in the midperiphery. The

TABLE I. Tissue Hexosamine Content and Urinary Excretion of Bound Hexamine in Glucosamine-Treated Rats.

	Hexosamine content (μg/g tissue)		Urinary excretion of bound hexosamine (μg/day)
	Liver	Kidney	
Normal	831 ± 36	1410 ± 128(6)	499 ± 68(11)
Glucosamine-treated	956 ± 62	1780 ± 137(6)	768 ± 102*(9)

Mean ± SE.
*$P < 0.05$, compared to normal.
Number of animals in parentheses

endothelial cells (E) and mural cells (M) were differentiated at × 400 magnification and ratios (E/M) were derived after counting all capillary cells in four pictures for each rat (3).

Results. Intraperitoneal glucosamine injections did not induce hyperglycemia in rats, nor did they alter significantly the hexosamine content of liver and kidney. A significant elevation of urinary bound hexosamine was noted in the glucosamine treated rats, but this was smaller than that previously noted in diabetic rats (1) (Table I). The oral glucose tolerance curves (Fig. 1A) did not show significant differences until the third hour when a glucose elevation was noted in the glucosamine treated rats. As can be seen in Fig. 1B, the insulinogenic response to glucose was negligible in the glucosamine treated animals even

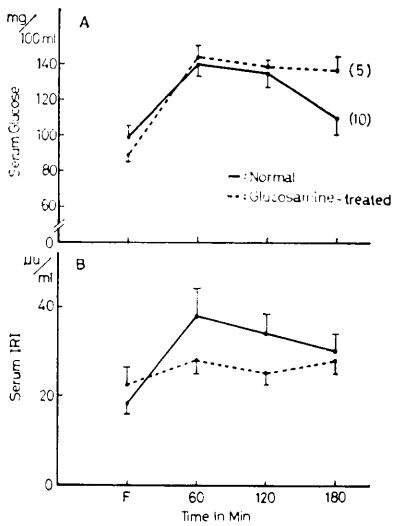


FIG. 1. Glucose (A) and immunoreactive serum insulin (B) responses to oral glucose (3 g/kg). Mean ± SE. Number of animals in parentheses.

though the fasting serum IRI level was not significantly different from normal. Treatment with glucosamine or streptozotocin caused a significant reduction in liver glucokinase activity (Table II) (when expressed per 100 g body wt, the decrease noted in glucosamine-treated rats was not statistically significant). On the other hand, G6P-ase activity per 100 g body wt was significantly increased in both the diabetic and in the glucosamine animals, although the magnitude of the increase was much smaller in the latter. The pancreas of the glucosamine treated rats showed a minimal increase in β cell granularity (Fig. 2C) compared to that of the normal rats (Fig. 2A), whereas the β cells were markedly degranulated in the diabetic rats (Fig. 2B). There were no remarkable histological differences between the livers and kidneys of the three groups of rats.

The glucosamine treated and the diabetic rats showed a significant increase in urinary protein excretion (Table III).

No microaneurysms were detected in the retinal capillaries of either the diabetic or the glucosamine treated rats (Fig. 3B and 3C), although a few capillaries showed possible occlusive lesions (3B) and acellularity (3C). Increased E/M ratios due to a significant decrease in numbers of mural cells, were found in both groups as compared to the controls (Table IV).

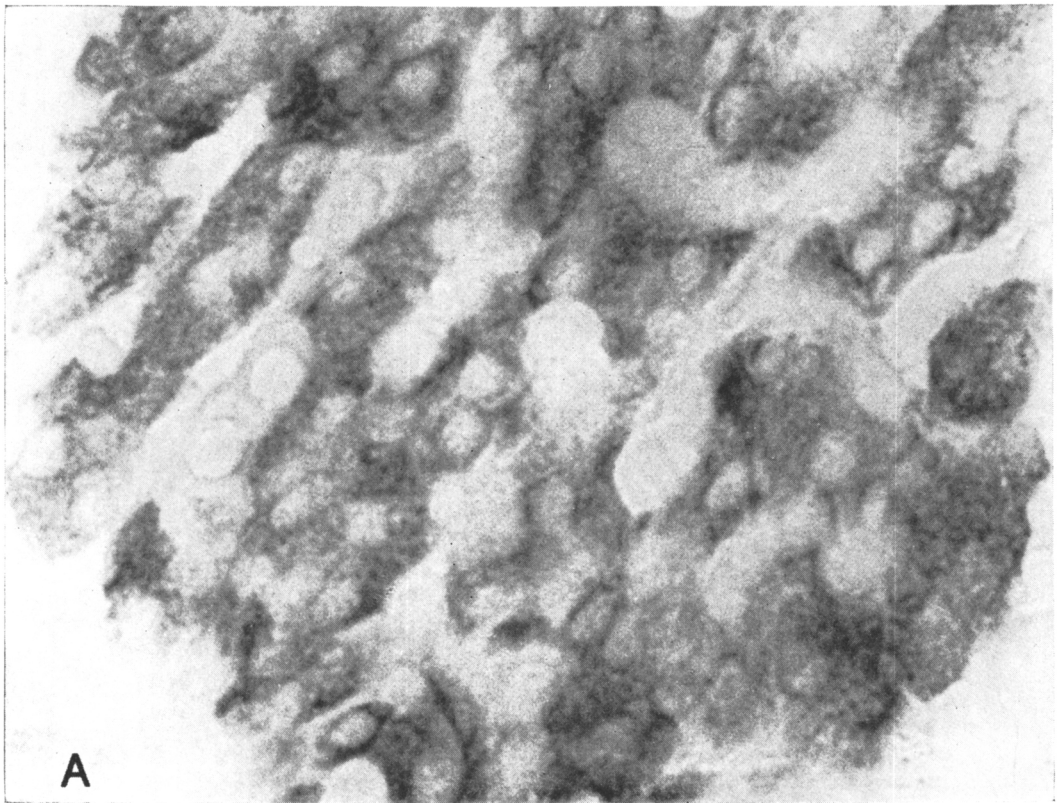
Discussion. The administration of large amounts of glucosamine produces hyperglycemia (11). The dose used in our experiments caused only a mild impairment of glucose tolerance, accompanied by a decrease in the insulin response to glucose and by an increase in granularity of the pancreatic β cells. The metabolic abnormality and the apparent sup-

TABLE II. Liver Glucokinase and G6Pase Activities of Normal, Diabetic, and Glucosamine Treated Rats.

	Glucokinase		G6Pase	
	μmoles NADPH formed/hr/g tissue	μmoles NADPH formed/hr/100g body wt	mmoles Phosphate formed/hr/g tissue	mmoles Phosphate formed/hr/100g body wt
Normal	129 ± 4.3	348 ± 15.9(11)	15.1 ± 0.7	37.6 ± 2.4(9)
Diabetic	12.7 ± 5.1*	51.9 ± 20.9(12)*	16.3 ± 2.2	72.0 ± 10.7(6)**
Glucosamine-treated	99.6 ± 9.2*	297 ± 27.8(13)	16.7 ± 0.4	45.4 ± 2.0(9)***

*$P < 0.005$; **$P < 0.01$; ***$P < 0.05$, compared to normal.
Number of animals in parentheses.

pression of insulin release could be attributed to the inhibitory effect of glucosamine on hexokinase activity (6, 12–14). In addition, in the glucosamine treated rats we noted a decrease in the number of mural cells in the retinal capillaries. A similar decrease was noted in the streptozotocin treated rats, although other histologic changes were minimal and no microaneurysms were detected. These observations are consistent with the retinal microangiopathy observed by other investigators in diabetic animals (3,15). The glucosamine treated as well as the streptozotocin diabetic rats showed an increase in the urinary excretion of bound hexosamine and of protein. The results of our experiment suggest that glucosamine may produce retinal vascular changes similar to those seen in diabetic rats even in the absence of significant hyperglycemia. The presence of proteinuria in both groups of animals suggests but, of course, does not prove that the renal glomeruli may also



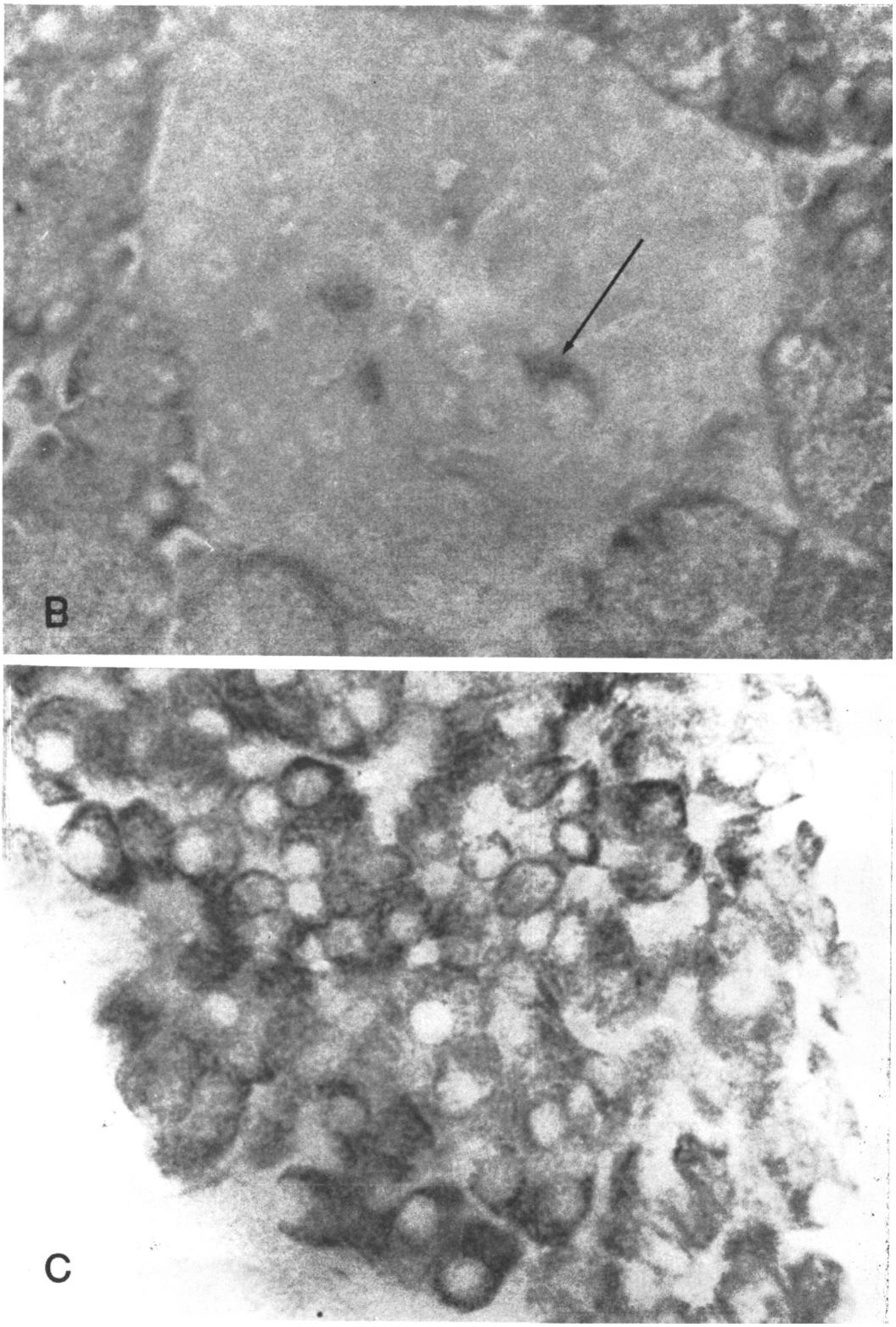


FIG. 2. Pancreatic islet of a normal (A), a diabetic (B), and a glucosamine-treated (C) rat. Aldehyde fuchsin. The arrow in (B) points to one of the few remaining granulated β cells.

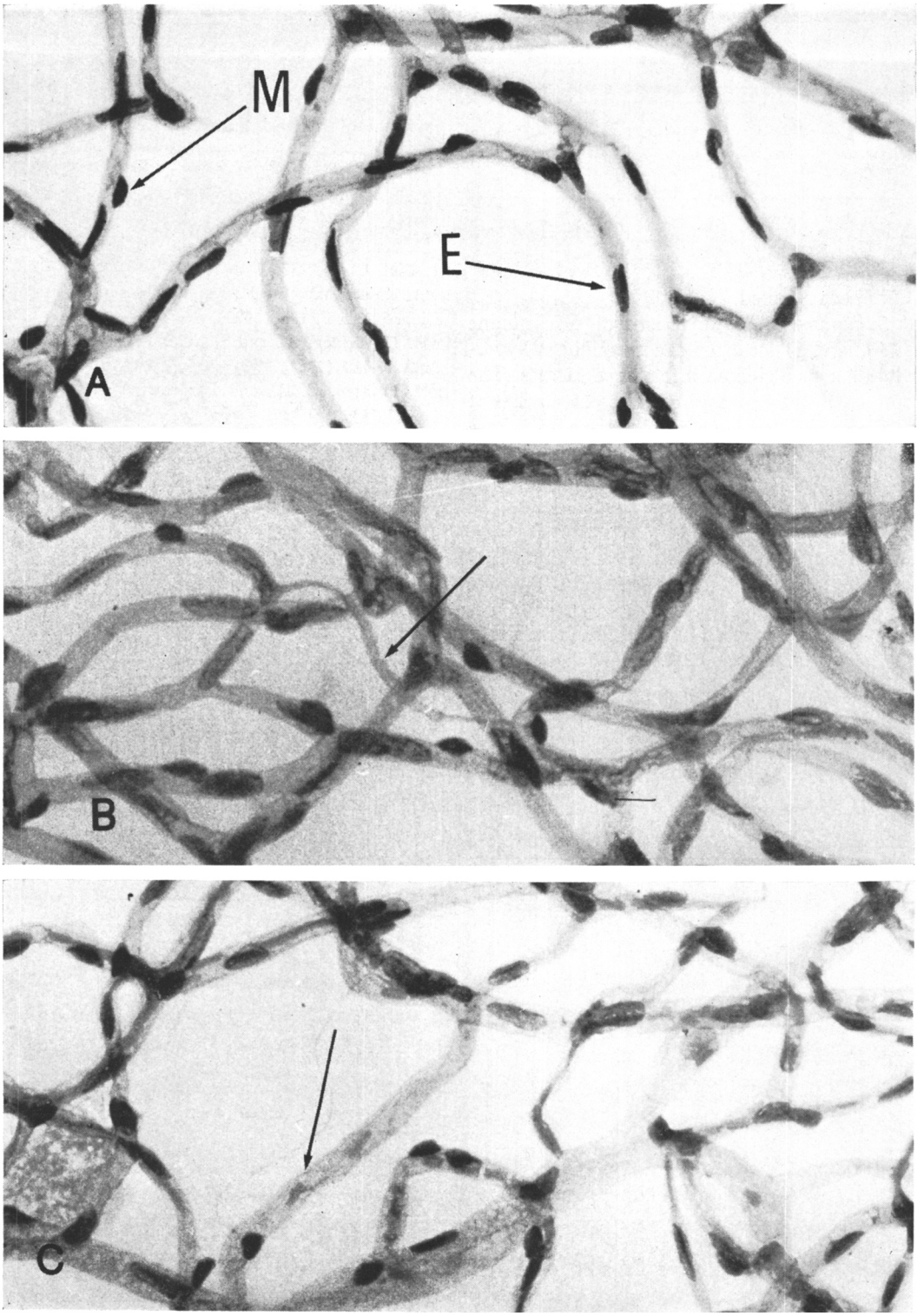


FIG. 3. Trypsin digested flat retinal preparations of a normal (A), a diabetic (B), and a glucosamine-treated rat (C), showing endothelial (E) and mural (M) cells. The arrow points to retinal capillaries showing a possible occlusion and an acellular portion (C).

TABLE III. Urinary Protein Excretion in Normal, Diabetic and Glucosamine-treated Rats.

	mg Protein/day
Normal	19.3 ± 2.6(8)
Diabetic	37.6 ± 2.3(8)*
Glucosamine-treated	40.2 ± 4.0(6)*

Number of animals in parentheses.
*$P < 0.005$, compared to normal.

have been affected.

Summary. Daily injections of glucosamine (250 mg/kg) for 2 mo caused minimal hyperglycemia and glucose intolerance in rats. The activity of liver glucokinase decreased; that of G6P-ase did not change significantly. There was a slight increase in the granularity of the pancreatic β cells, accompanied by a decreased insulin response to a glucose load. The excretion of protein and bound hexosamine in the urine was elevated. The number of mural cells in the retinal capillaries was decreased. These results are consistent with the hypothesis that increased glucosamine synthesis in diabetes may be related to the development of microangiopathy.

TABLE IV. Ratio of Endothelial Cells to Mural Cells (E/M) of the Retinal Capillaries of Rats.

	E/M ratio
Normal	1.95 ± 0.05(6)
Diabetic	2.29 ± 0.07(6)*
Glucosamine-treated	2.35 ± 0.05(8)*

Mean ± SE.
*$P < 0.05$, compared to normal.
Number of animals in parentheses.

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