Interaction of Ascorbic Acid and Glucose on Production of Collagen and Proteoglycan by Fibroblasts

ELIZABETH FISHER, SUSAN V. McLENNAN, HISAYA TADA, SCOTT HEFFERNAN, DENNIS K. YUE, AND JOHN R. TURTLE

Collagen and proteoglycans are two major constituents of the extracellular matrix, and their abnormalities have been incriminated in the pathogenesis of diabetic complications. A decrease of plasma ascorbic acid has been reported in diabetes and thus may play a role in the collagen and proteoglycan abnormalities in diabetes. Ascorbic acid and glucose share structural similarity, and their metabolism may interact at the level of membrane transport and cellular action. In this study, we used a fibroblast culture system to explore this possibility. Ascorbic acid increased collagen and proteoglycan both in the culture medium and the cell layer. This stimulatory action of ascorbic acid was inhibited by the presence of glucose at a concentration of 25 mM. The effect of high glucose concentration was not mediated by inhibition of ascorbic acid uptake by fibroblasts. Insulin is able to abolish this inhibitory action of glucose on collagen production, but the precise mechanism is unclear. These results show that the high glucose concentration in diabetes can impair the action of ascorbic acid at the cellular level. This may further accentuate the problem of decreased availability of this vitamin as a result of its low plasma concentration. *Diabetes* 40:371–76, 1991

Abnormalities in the metabolism of ascorbic acid have been reported in diabetes (1–5). Ascorbic acid is an important cofactor in the synthesis of collagen and proteoglycan (6–8). These two classes of extracellular matrix proteins play major roles in determining the properties and functions of many vital structures, including the blood vessels, granulation tissue, and basement membrane (9–12). In addition, proteoglycan associated with cell membranes regulates growth and cell-cell interaction (13–16). Many abnormalities of collagen and proteoglycan have been described in diabetes and may be responsible for some of the tissue damage seen in diabetes (10–12,17,18). In view of the relationship between ascorbic acid, collagen, and proteoglycan metabolism, it is possible that the disturbance of ascorbic acid homeostasis in diabetes may play a pathogenetic role in some diabetic complications.

A decrease in the plasma concentration of ascorbic acid has been observed in both animals and humans with diabetes (1–5). This suggests the possibility of reduced availability of this vitamin to fulfill its various biological functions. The uptake of ascorbic acid into the cell is mediated by processes related to glucose transport and has been shown to be inhibited by glucose (19–21). Thus, there is the potential that the high extracellular glucose concentration in diabetes may further impair cellular uptake of ascorbic acid and accentuate any problem associated with its deficiency. In this study, we explored this possibility with a fibroblast culture system to examine the interaction of glucose and ascorbic acid on collagen and proteoglycan metabolism.

RESEARCH DESIGN AND METHODS

[^{35}S]sulfuric acid (98%), L-[carboxyl-^{14}C]ascorbic acid, and L-[2,3-^{3}H]proline were obtained from Amersham (Aylesbury, UK). Heparin, trypsin, soybean trypsin inhibitor, beef insulin, cetylpyridinium chloride, *N*-ethylmaleimide, phenylmethylsulfonyl fluoride, ε-amino-*n*-caproic acid, benzamidine HCl, collagenase type VII, chondroitinase ABC, and heparinase were obtained from Sigma (St. Louis, MO). Basal modified Eagle's (BME) culture medium was purchased from Commonwealth Serum Laboratories (Melbourne, Australia) and fetal calf serum (FCS) from Cytosystems (Castle Hill, Sydney, Australia). Fluorescamine was purchased from Roche (Basel) and 3MM filter paper from Whatman (Mainstone, UK). Culture flasks were purchased from Corning (Corning, NY).

From the Department of Medicine, The University of Sydney, Sydney, Australia.

Address correspondence and reprint requests to Dr. Dennis K. Yue, Department of Medicine, The University of Sydney, Sydney, NSW 2006, Australia.

Received for publication 23 January 1990 and accepted in revised form 7 November 1990.

Fibroblasts from a normal human neonatal foreskin were cultured in BME medium supplemented with 10% FCS and used between the 4th and 12th passage. The cells were cultured in 25-cm^2 flasks for the determination of collagen in the medium. For the measurement of collagen in the cell layer and proteoglycan in the medium and cell layer, fibroblasts were cultured in six-well plates. In selected experiments, to determine the effects of glucose and ascorbic acid on fibroblast growth, the DNA content of each well was measured by fluorometry with calf thymus DNA as standard.

Radioactive labeling of collagen and proteoglycan was required for their quantitation except in the case of collagen in the culture medium, which was present in sufficient concentration to enable measurement of hydroxyproline by high-performance liquid chromatography (HPLC). To label cell layer collagen, confluent fibroblasts were incubated in FCS-free medium containing 10 μCi/ml of L-[2,3-^{3}H]proline for 24 h before study. To label proteoglycan, confluent fibroblasts were incubated in medium containing 12.5 μCi/ml of ^{35}S for 18 h.

At confluence, fibroblasts were washed twice in phosphate-buffered saline (PBS; 140 mM NaCl, 3 mM KCl, 0.3 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.6). The cells were divided into groups of four to six flasks. Each flask was incubated in 2 ml of medium containing either 5 or 25 mM glucose, with or without 0.56 mM ascorbic acid. To prevent further cell division, FCS was not added to the medium. After 24 h, the medium was harvested, and the proteins were precipitated by 10% trichloroacetic acid. Washing the precipitate twice in ether removed the trichloroacetic acid, and excess ether was then removed by evaporation. The medium was hydrolyzed for 18 h at 110°C in 6 N HCl. The collagen content of the hydrolysate was quantitated by the measurement of hydroxyproline with a Pico-Tag HPLC amino acid column (Waters, Milford, MA). Results were expressed as nanomoles of hydroxyproline per flask. The protein content of the medium was quantitated by the measurement of amino acids in the hydrolysate with fluorescamine, and results were expressed as micrograms of amino acid per flask.

To test the effects of insulin, the same protocol that was used above was used with additional flasks of fibroblasts incubated in the presence of insulin at a final concentration of 3×10^{-7} mM.

After the labeling of confluent fibroblasts with L-[2,3-^{3}H]-proline, the medium was removed, and the cell layer was washed three times in PBS. Cells were detached by scraping with a rubber policeman, further centrifuged, washed twice with PBS, and then resuspended in 1 ml PBS. The cell suspension was then sonicated and the protein precipitated and washed three times with trichloroacetic acid at a final concentration of 10%. The protein precipitate was then solubilized in 600 μl 0.2 N NaOH, and the collagen and noncollagenous protein were quantitated by the release of radioactivity after specific collagenase digestion as described by Peterkofsky and Diegelman (22). Results were expressed as disintegrations per minute per well.

Release of ^{35}S-labeled proteoglycan and glycosaminoglycan into the culture medium was studied under the same incubation conditions (±0.56 mM ascorbic acid, 5 or 25 mM glucose) as described above. After 18 h of incubation, the medium was harvested, and duplicate aliquots of 100 μl were spotted onto filter paper (3MM) impregnated with 2.5% cetylpyridinium chloride, which served to precipitate the highly charged proteoglycans and glycosaminoglycans (23). Other sulfated proteins and free [^{35}S]sulfuric acid were washed away by sodium sulfate (25 mM) and water before the paper was dried and counted. To determine the radioactivity specifically incorporated into proteoglycan (rather than glycosaminoglycan), the filter paper was shaken for an additional 2 h in 20% trichloroacetic acid to precipitate proteoglycan and release the low–molecular-weight glycosaminoglycan. Results were expressed as DPM per flask.

The proteoglycan in the culture medium was characterized by the technique of Robinson and Gospodarowicz (24). Briefly, the labeled proteoglycan was separated from free ^{35}S by Sephadex G-50 chromatography. The radioactive peak present in the void volume was applied onto a DEAE-Sephacel column and eluted with a gradient of 0.1–1.2 M NaCl. This separated proteoglycan from other labeled molecules. The proteoglycan was then incubated with papain to release glycosaminoglycan, which was then characterized by digestion with either chondroitinase ABC or heparinase.

Confluent fibroblasts were washed four times in BME medium containing protease inhibitors (10 mM *N*-ethylmaleimide, 5 mM phenylmethylsulfonyl fluoride, 0.1 mM ε-amino-*n*-caproic acid, 5 mM benzamidine HCl) and then solubilized in 250 μl of 1% Triton X-100. The solubilized product was loaded onto cetylpyridinium chloride–impregnated filter paper, and the proteoglycan and glycosaminoglycan content was measured as described for the medium.

After removal of the medium, the fibroblasts were washed twice in medium containing protease inhibitors. The cells were then scraped from the well and washed once more in medium containing protease inhibitors. Membrane-associated proteoglycans were then released sequentially by incubation of the cells in 1 ml of heparin (1 mg/ml) and trypsin (50 μg/ml) at 37°C for 30 min (25,26). The action of trypsin was stopped by adding 500 μl soybean trypsin inhibitor (1 mg/ml). A 500-μl aliquot of each supernatant was counted, and the results were expressed as disintegrations per minute released per well.

The transport of ascorbic acid into fibroblasts was studied by the uptake of L-[carboxyl-^{14}C]ascorbic acid. At the beginning of the experiment, the medium was changed to one containing 25 μl of L-[carboxyl-^{14}C]ascorbic acid (25 μCi/ml) in either 5 or 25 mM glucose. At various time points (2, 10, 15, and 30 min and 24 h), the medium was removed and the cells placed on ice before being washed three times with ice-cold PBS. The cells were solubilized in 500 μl of 1% sodium dodecyl sulfate, and an aliquot of 400 μl was then counted to determine the intracellular accumulation of L-[carboxyl-^{14}C]ascorbic acid.

Results are expressed as means ± SD and were compared by analysis of variance with post hoc Duncan's multiple range test.

RESULTS

Production of collagen by confluent fibroblasts was increased 5.6-fold by ascorbic acid. This stimulatory action of ascorbic acid was substantially inhibited by glucose at a concentration of 25 mM. Measurement of amino acid concentration in the incubation medium showed that protein

production was increased 2.6-fold by ascorbic acid; this was almost completely abolished by the presence of high glucose concentration. In the absence of ascorbic acid, glucose had no demonstrable effect on the production of collagen or protein by fibroblasts (Fig. 1). Ascorbic acid increased the hydroxyproline–amino acid ratio from a basal value of 0.032 to 0.071; this was not suppressed when 25 mM of glucose was present in the medium (0.078). The actions of ascorbic acid and glucose were not due to a change in cell number because they did not affect the DNA content (control 33.4 ± 2.2 μg/ml, high glucose 36.6 ± 2.5 μg/ml, ascorbic acid 38.4 ± 5.6 μg/ml, glucose + ascorbic acid 36.6 ± 2.8 μg/ml).

In a second experiment, the same pattern of interaction between ascorbic acid and glucose on fibroblast function was again demonstrated. Insulin alone had no effect on collagen or protein production but was able to preserve the stimulatory effects of ascorbic acid in the presence of a high glucose concentration (Table 1).

TABLE 1
Effects of ascorbic acid, glucose, and insulin on production of collagen and protein by fibroblasts

Incubation medium	Hydroxyproline (nM/flask)	Amino acid (μg/flask)
Control medium	2.6 ± 2.9	42.5 ± 21.2
With addition of ascorbic acid	25.2 ± 17.4	82.4 ± 26.9
With addition of insulin	1.8 ± 2.9	34.1 ± 3.5
With addition of ascorbic acid, glucose, and insulin	22.5 ± 14.8	97.6 ± 18.7

Ascorbic acid was present at concentration of 0.56 mM, glucose at 25 mM, and insulin at 3×10^{-7} M. Values are means ± SD.

As shown in Fig. 2, a high glucose concentration (25 mM) was also able to inhibit the ascorbic acid–induced stimulation of collagen production within the cell layer.

Ascorbic acid and glucose affected proteoglycan production by fibroblast in a manner similar to their effects on collagen. In the presence of ascorbic acid, the release of proteoglycans and glycosaminoglycans by fibroblasts into the medium was greatly increased. This stimulatory action was completely abolished by a high glucose concentration (Fig. 3). The 25 mM of glucose also inhibited the stimulation of proteoglycan production by ascorbic acid within the cell layer (Fig. 3*B*). By contrast, neither ascorbic acid nor glucose had any effect on the ^{35}S radioactivity released by heparin or trypsin from fibroblast cell membranes (Fig. 4).

Treatment of cetylpyridinium chloride precipitate of the medium with trichloroacetic acid showed that 22.1 ± 2.2% of the radioactivity was in the form of proteoglycan, the balance being glycosaminoglycan. The proportion was not changed by ascorbic acid or glucose. Further characteriza-

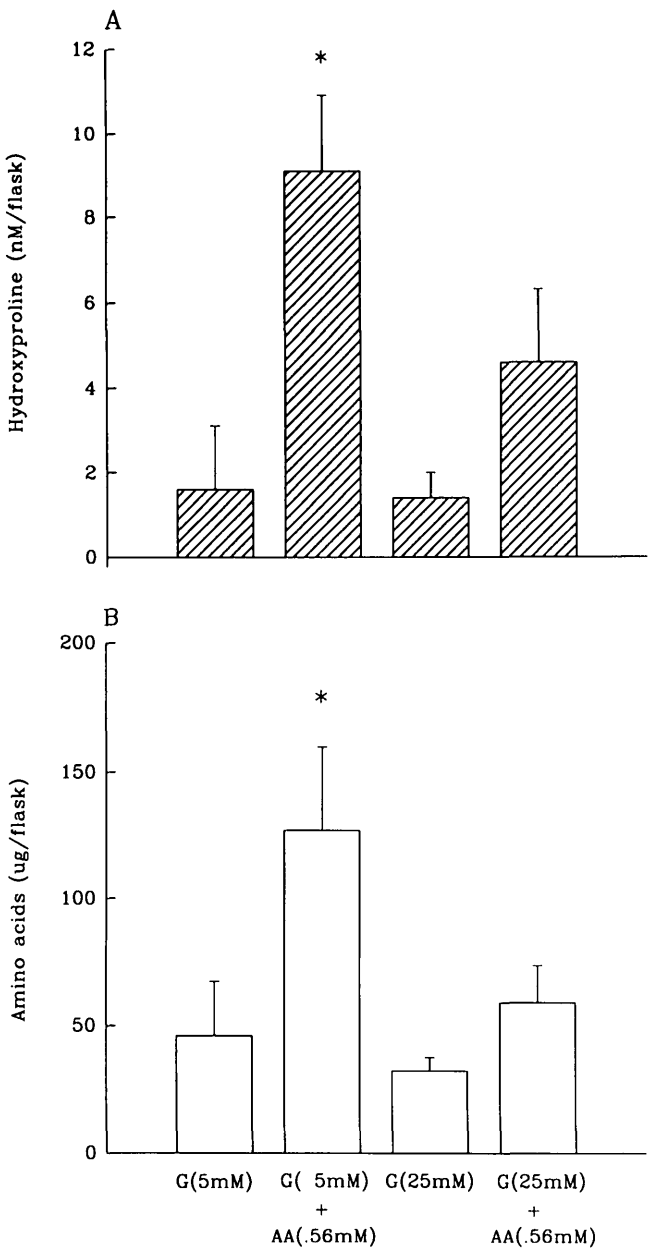

FIG. 1. Effects of ascorbic acid (AA) and glucose on collagen (*A*) and amino acid (*B*) production by fibroblasts. *$P < 0.001$ vs. cells incubated in 5 mM glucose.

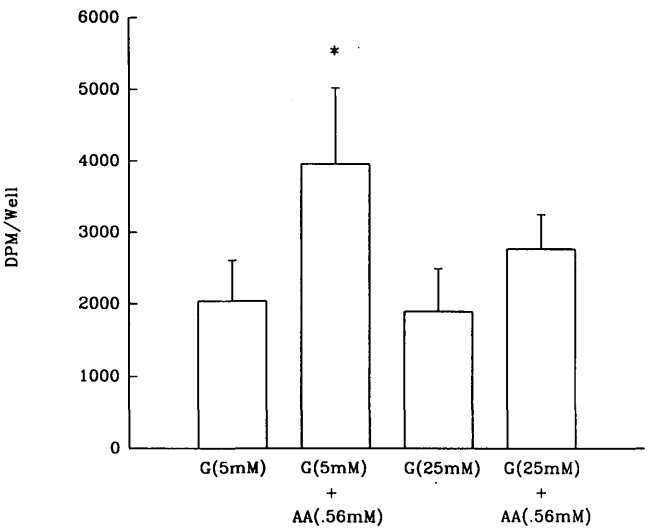

FIG. 2. Effects of ascorbic acid (AA) and glucose (G) on collagen content of fibroblast cell layer. DPM, disintegrations/min. *$P < 0.05$ vs. cells incubated in 5 mM glucose.

tion showed that 46% of the total glycosaminoglycan was degraded by chondroitinase ABC and 52% by heparinase.

The uptake of L-[carboxyl-^{14}C]ascorbic acid by fibroblasts at various time points is shown in Table 2. At 2 min, ascorbic acid uptake was 36% lower in the presence of high glucose concentration. At 24 h, the corresponding figure was only 12%.

DISCUSSION

A decrease in the plasma concentration of ascorbic acid in diabetes has been reported previously (1–5). The reduction occurs regardless of a species' ability to synthesize this vitamin because it has been observed in both humans and rats. The precise mechanism underlying this decrease of ascorbic acid in diabetes is unclear, but it has been found, at least in the rats, to be related to the polyol pathway because it can be prevented by the administration of aldose reductase inhibitors and *myo*-inositol (2,3).

Ascorbic acid has many important biological functions, and its deficiency has been postulated to be important in the pathogenesis of some diabetic complications (19,21). Ascorbic acid is an antioxidant, and its depletion may accentuate oxidative damage leading to secondary effects on many cellular structures and functions (6,27). Ascorbic acid also stimulates the synthesis of collagen and proteoglycan, which are both important constituents of tissue and are widely distributed in the body (8,9). In recent studies of diabetic complications, considerable attention has focused on the abnormalities of these two classes of extracellular matrix proteins. They include the increase in biochemical strength of collagen fibers, the impairment of granulation tissue formation, and the decrease of proteoglycan in basement membrane (10–12,28). These changes could in turn have profound effects on the physical properties of blood vessels, the process of wound healing, and the filtration characteristics of glomeruli. Although it remains unproved, there is reason to believe that ascorbic acid deficiency may play a role in the pathogenesis of diabetic complications.

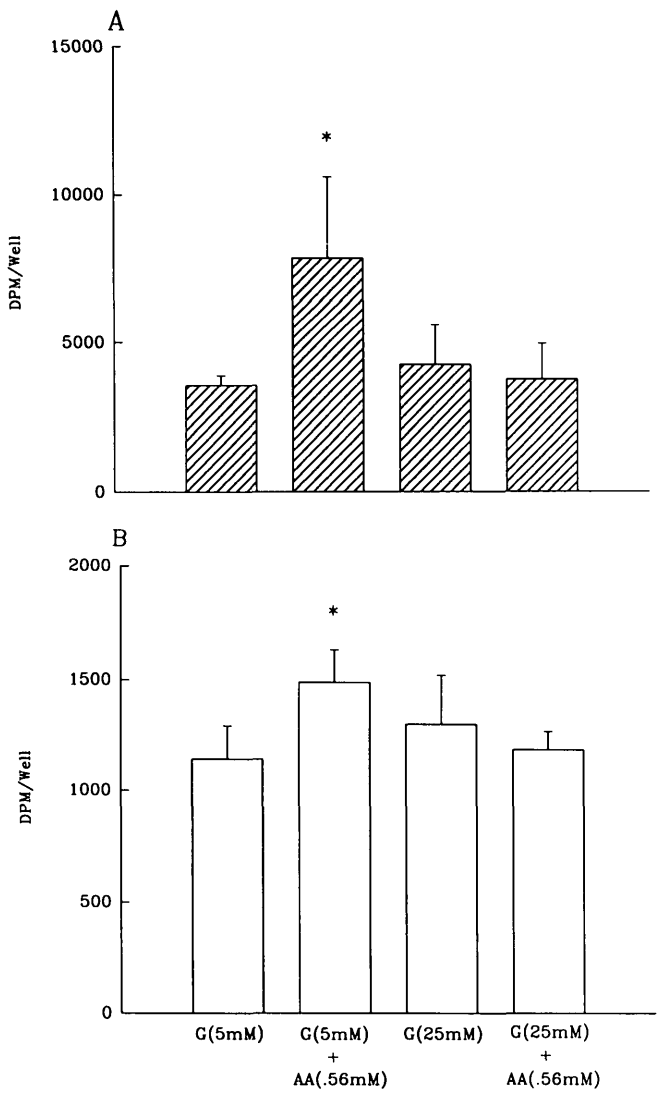

FIG. 3. Effects of ascorbic acid (AA) and glucose (G) on proteoglycan in culture medium (*A*) and fibroblast cell layer (*B*). DPM, disintegrations/min. *$P < 0.05$ vs. cells incubated in 5 mM glucose.

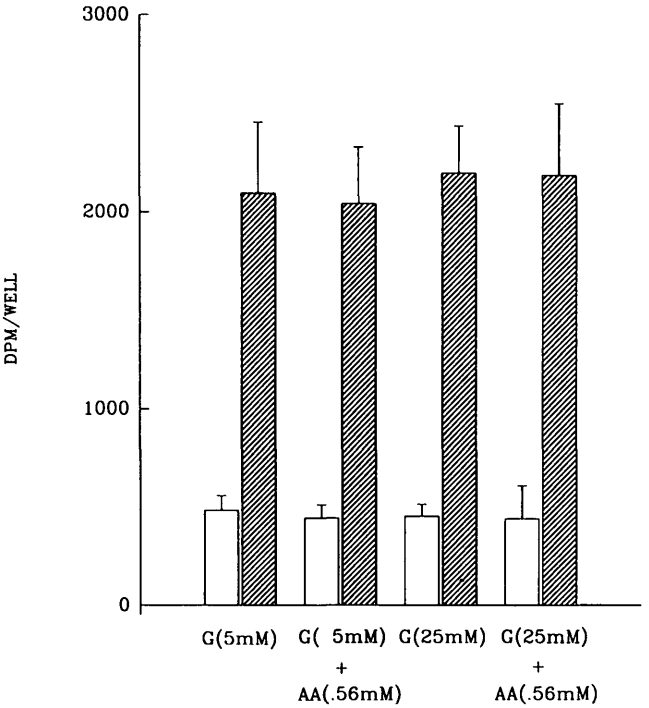

FIG. 4. Effects of ascorbic acid (AA) and glucose (G) on heparin-displaceable (*open bars*) and trypsin-releasable (*hatched bars*) fractions of cell-associated proteoglycans. DPM, disintegrations/min.

TABLE 2
Effects of high glucose concentration on fibroblast uptake of L-[carboxyl-^{14}C]ascorbic acid

	L-[carboxyl-^{14}C]ascorbic acid uptake	
Time	Glucose (5 mM)	Glucose (25 mM)
2 min	3571 ± 828	2298 ± 954
10 min	2888 ± 134	2621 ± 591
15 min	4585 ± 307	4002 ± 678
30 min	8645 ± 773	7737 ± 842
24 h	12,548 ± 2993	11,061 ± 3636

Values are means ± SD.

This study confirms the dependence of fibroblasts on ascorbic acid for production of collagen and proteoglycans. Thus, the reduction of ascorbic acid level in diabetes could in its own right affect the synthesis of the two classes of matrix protein. Our results show that this situation can be further aggravated by the presence of hyperglycemia, which impairs the action of ascorbic acid.

Ascorbic acid increases the release of both hydroxyproline and amino acid into the culture medium, but its effect is greater on the former, indicating a preferential action on collagen production. By contrast, a high glucose concentration reduces collagen and protein synthesis equally, thus the hydroxyproline–amino acid ratio remains elevated when ascorbic acid and high glucose concentrations are present together. Although we have been able to show that a high glucose concentration causes a slight inhibition of ascorbic acid uptake in the early phase, as reported by others (19–21), the accumulation of ascorbic acid by fibroblasts over the 24 h of the experiment was only slightly suppressed. From our dose-response curve (results not shown), this reduction in ascorbic acid uptake cannot explain the fall in collagen and proteoglycan production. Thus, 25 mM of glucose does not produce these results solely by interfering with the availability of ascorbic acid to the fibroblasts.

Many conflicting results on the effects of high glucose concentrations have been reported in the literature. High glucose concentration has been shown to stimulate collagen and proteoglycan synthesis of basement membrane producing Engelbreth-Holm-Swarm tumor. In cultured chondrocytes and fibroblasts, high glucose concentration has been reported to have no stimulatory action on proteoglycans (29–31). In addition, an increase or no change in collagen production has been reported in fibroblasts cultured in a high glucose concentration (32,33). These differences may be due to variations in methodology and the types of cells studied. In our study, the effects of glucose on collagen and proteoglycans are the same in the medium and the cell layer, suggesting that what happens in the medium is a reflection of intracellular events. Whatever the mechanism, the ability of glucose to reduce the synthesis of some components of the extracellular matrix may be an important component of the general phenomenon of glucose toxicity (30). Note that the consequence of high glucose concentration is different when insulin is present. In insulin-dependent and non-insulin-dependent diabetes, the same degree of hyperglycemia can be present under vastly different insulin concentrations. Perhaps these patients will also react to the combination of ascorbic acid deficiency and hyperglycemia differently.

Studies on the disturbance of proteoglycan metabolism in diabetes have traditionally focused on its function as an extracellular matrix protein such as in the maintenance of the charge and permeability properties of basement membrane (10–12). It is less well appreciated that the intracellular and membrane-associated proteoglycans are also carefully regulated and have important functions in the control of cell growth and cell-cell interaction. Whether the metabolism of these cell-associated proteoglycans is abnormal in diabetes is largely unknown. In contrast to the findings in the medium and cell layer, glucose and ascorbic acid have no effect on either the heparin-displaceable or trypsin-releasable forms of membrane-bound proteoglycan. This indicates that the observed changes of proteoglycan concentration in the culture medium are not due to the release of these specific types of proteoglycans from the membrane site. Although no effect of glucose or ascorbic acid on fibroblast cell membrane proteoglycan is demonstrable, different cell types may behave differently, and each will need to be investigated separately.

Whether ascorbic acid deficiency plays a significant role in the pathogenesis of diabetic complications is unknown. This study reveals the complex interaction between glucose and ascorbic acid on cellular function. To evaluate the importance of vitamin C in diabetes, consideration must be paid to its interrelationship with glucose and insulin metabolism.

ACKNOWLEDGMENTS

This study was supported by the National Health Medical Research Council of Australia and by a grant from the Wyeth-Ayerst Company.

REFERENCES

1. Som S, Basu D, Deb S, Choudhury PR, Mukherjee S, Chatterjee SN, Chatterjee IB: Ascorbic acid metabolism in diabetes mellitus. *Metabolism* 30:572–77, 1981
2. Yue DK, McLennan S, Fisher E, Heffernan S, Capogreco C, Ross GR, Turtle JR: Ascorbic acid metabolism and polyol pathway in diabetes. *Diabetes* 38:257–61, 1989
3. McLennan S, Yue DK, Fisher E, Capogreco C, Heffernan S, Ross GR, Turtle JR: Deficiency of ascorbic acid in experimental diabetes: relationship with collagen and polyol pathway abnormalities. *Diabetes* 37:359–61, 1988
4. Chen MS, Hutchinson ML, Pecoraro RE, Lee WYL, Labbé RF: Hyperglycemia-induced intracellular depletion of ascorbic acid in human mononuclear leukocytes. *Diabetes* 32:1078–81, 1983
5. Stankova L, Riddle M, Larned J, Burry K, Menashe D, Hart J, Bigley R: Plasma ascorbate concentrations and blood cell dehydroascorbate transport in patients with diabetes mellitus. *Metabolism* 33:347–53, 1984
6. Levine M: New concepts in the biology and biochemistry of ascorbic acid. *N Engl J Med* 314:892–902, 1986
7. Barnes MJ: Function of ascorbic acid in collagen metabolism. *Ann NY Acad Sci* 258:264–75, 1976
8. Vertlangieri AJ, Stevens JW: L-Ascorbic acid: effects on aortic glycosaminoglycans ^{35}S incorporation in rabbit atherogenesis. *Blood Vessels* 16:177–85, 1979
9. *Extracellular Matrix Biochemistry*. Piez KA, Reddie AF, Eds. New York, Elsevier, 1984
10. Klein DJ, Oegema TR Jr, Brown DM: Release of glomerular heparan-$^{35}SO_4$ proteoglycan by heparin from glomeruli of streptozocin-induced diabetic rats. *Diabetes* 38:130–39, 1989
11. Cohen MP, Klepser H, Wu V-Y: Undersulfation of glomerular basement membrane heparan sulfate in experimental diabetes and lack of correction with aldose reductase inhibition. *Diabetes* 37:1324–27, 1988
12. Rohrbach DH, Hassell JR, Kleinman HK, Martin GR: Alterations in the basement membrane (heparan sulfate) proteoglycan in diabetic mice. *Diabetes* 31:185–88, 1982
13. Hook M: Cell-surface glycosaminoglycans. *Annu Rev Biochem* 53:847–69, 1984
14. Yamada KM: Cell surface interactions with extracellular materials. *Annu Rev Biochem* 52:761–99, 1983
15. Ruosslahti E: Proteoglycans in cell regulation. *J Biol Chem* 264:13369–72, 1989
16. Iozzo RV: Cell surface heparan sulfate proteoglycan and the neoplastic phenotype. *J Cell Biochem* 37:61–78, 1988
17. Yue DK, Swanson B, McLennan S, Marsh M, Spalivero J, Delbridge L, Reeve T, Turtle JR: Abnormalities of granulation tissue and collagen formation in experimental diabetes, uremia and malnutrition. *Diabetic Med* 3:221–25, 1986
18. Spanheimer RG, Umpierrez GE, Stumpf V: Decreased collagen production in diabetic rats. *Diabetes* 37:371–76, 1988
19. Kapeghian JC, Verlangieri AJ: The effects of glucose on ascorbic acid uptake in heart endothelial cells: possible pathogenesis of diabetic angiopathies. *Life Sci* 34:577–84, 1984
20. Khatami M, Li W, Rockey JH: Kinetics of ascorbate transport by cultured retinal capillary pericytes. *Invest Ophthalmol Visual Sci* 27:1665–71, 1986
21. Bigley R, Wirth M, Layman D, Riddle M, Stankova L: Interaction between

glucose and dehydroascorbate transport in human neutrophils and fibroblasts. *Diabetes* 32:545–48, 1983
22. Peterkofsky B, Diegelman R: Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. *Biochemistry* 10:988–94, 1971
23. Rapraeger A, Bernfield M: Cell surface proteoglycan of mammary epithelial cells. *J Biol Chem* 260:4103–109, 1986
24. Robinson J, Gospodarowicz D: Glycosaminoglycans synthesized by cultured bovine corneal endothelial cells. *J Cell Physiol* 117:366–78, 1983
25. Kjellen L, Olberg A, Hook M: Cell surface heparan sulfate. *J Biol Chem* 255:10407–13, 1980
26. Yanagishta M, Hascall VC: Metabolism of proteoglycans in rat ovarian granulosa cell culture: multiple intracellular degradative pathways and the effect of chloroquine. *J Biol Chem* 257:10270–83, 1984
27. Freeman BA, Crapo JD: Biology of disease: free radicals and tissue injury. *Lab Invest* 47:412–26, 1982
28. Andreassen TT, Seyer-Hanson K, Bailey AJ: Thermal stability, mechanical properties and reducible crosslinks of rat-tail tendon in experimental diabetes. *Biochim Biophys Acta* 677:313–17, 1981
29. Pihlajaniemi T, Myllyla R, Kiririkko KI, Tryggrason K: Effects of streptozotocin diabetes, glucose and insulin on the metabolism of type IV collagen and proteoglycan in murine basement membrane-forming EHS tumour tissue. *J Biol Chem* 257:14914–20, 1982
30. Ledbetter SR, Wagner CW, Martin GR, Rohrbach DH, Hassell JR: Response of diabetic basement membrane–producing cells to glucose and insulin. *Diabetes* 36:1029–34, 1987
31. Stevens RL, Nissley PS, Kimura JH, Rechler MM, Caplan AI, Hascall VC: Effects of insulin and multiple-stimulating activity on proteoglycan biosynthesis in chondrocytes from the swarm rat sarcoma. *J Biol Chem* 256:2045–52, 1981
32. Villee DB, Powers ML: Effects of glucose and insulin on collagen secretion by human skin fibroblasts. *Nature* (Lond) 268:156–57, 1977
33. Smith BD, Silbert CK: Fibronectin and collagen of cultured skin fibroblasts in diabetes mellitus. *Biochem Biophys Res Commun* 100:275–82, 1981