ABNORMAL COLLAGEN IN CULTURES OF FIBROBLASTS FROM HUMAN BEINGS WITH DIABETES MELLITUS

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SUMMARY: Synthesis of collagen by fibroblasts from diabetic human beings was investigated to determine if abnormal synthesis might be responsible for an apparent acceleration of collagen aging observed previously in diabetics. Increased material was synthesized by cells from diabetics, and the material was abnormal in that it contained a very high molecular weight material and lacked a presumed procollagen dimer. Alpha chains did not appear altered. It is suggested that cultures of cells from diabetics yield a non-helical peptide of procollagen that contains abnormal reducible cross links and lacks normal non-reducible cross links.

In diabetes mellitus there is acceleration of a number of disease processes that are characteristic of aging and that occur in collagen-rich tissues (1,2). The most obvious and important of these is atherosclerosis. Collagen undergoes marked changes in properties with age that suggest increasing intermolecular cross linking. By the criterion of resistance to collagenase digestion, human collagen appears to have undergone accelerated aging in both juvenile-onset (3) and maturity-onset (2) diabetes. The collagen of rats with severe, prolonged alloxan-induced diabetes did not show altered susceptibility to collagenase digestion (2). This observation suggested that in human diabetes, an abnormal collagen might be synthesized, or collagen might be altered prior to, and independent of the carbohydrate abnormalities of diabetes.

This possibility was investigated by analyzing collagen synthesized by cultures of fibroblasts from individuals with diabetes. Such studies of normal collagen synthesis have shown that collagen is secreted from cells as a procollagen in which 3 pro α chains are held together by disulfide bonds in

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TABLE 1. Radioactive proline and glycine incorporation into medium proteins in 24 hours in 3 experiments, by confluent cultures of fibroblasts from diabetic and non-diabetic individuals

	Cell Origin	Passage No.	Days in Culture	CPM x 10^{-5}/culture
1	Diabetic - JC	11	6	3.75
	Diabetic - MB	11	6	3.04
	Control - RK	11	6	1.45
	Control - ST	11	6	1.70
2	Diabetic - MB	14	7	18.03
	Control - RK	14	7	13.28
3	Diabetic - JC	20	20	38.06
	Control - ST	20	20	26.74

non-helical register peptides. A peptidase then cleaves off the non-helical peptides, and the collagen molecules composed of three α chains aggregate to form fibers (4, 5).

MATERIALS AND METHODS: Cultures from two individuals with juvenile-onset diabetes were established: MB, a 22-year-old white male with diabetes diagnosed for 8 years, on daily insulin and JC, a 21-year-old white male with diabetes diagnosed 12 years previously, also on daily insulin. Original studies were with two healthy controls: ST, a 29-year-old white female and RK, a 51-year-old white male. Two additional healthy controls were subsequently added to the study: a 16-year-old white male and a 19-year-old white female.

Skin biopsies were obtained from the flexor surface of the forearm, minced, and washed in tissue culture medium containing Amphotericin B. Fragments were placed under cover slips in plastic dishes in Dulbecco-Vogt medium with 10% fetal calf serum and sodium ascorbate (75 µg/ml). When growth was established, cultures were trypsinized and cells were passed by inoculating 10^6 cells into 250 ml T flasks. Some cultures were frozen and stored in 7.5% dimethylsulfoxide in culture medium. Subsequent culture, labeling, and analytic procedures were identical to those of Goldberg and coworkers (4, 5). Briefly, confluent cultures were labeled with [^{3}H] glycine and proline for 24 hours. Soluble proteins in the media were partially purified by centrifugation and dialysis. Some samples were reduced with dithiothreitol and alkylated with iodoacetamide. Others were treated with pepsin to remove non-helical register peptides. All samples were lyophilized. Samples were heat denatured in urea and subjected to electrophoresis in SDS-5% acrylamide gels. Gels were sliced; swollen, eluted slices were counted in a toluene-based scintillant. Markers of

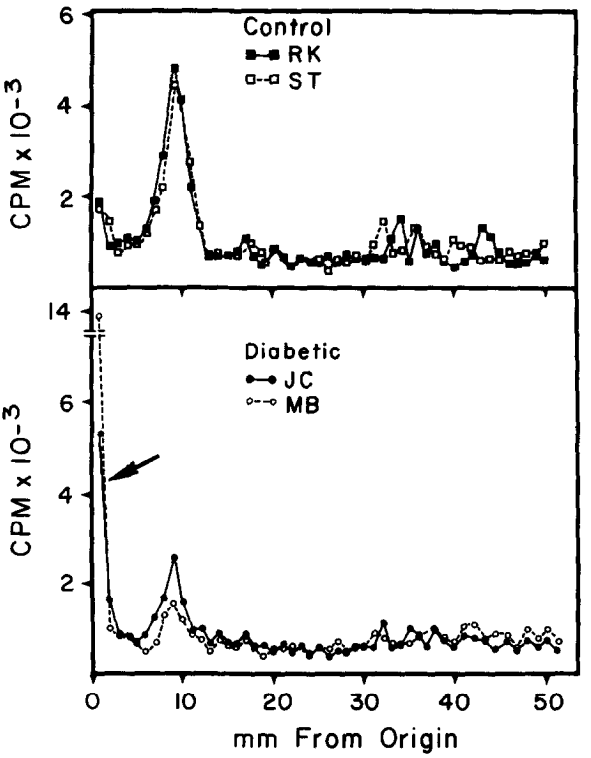

Fig. 1. Acrylamide gel electrophoresis of proteins synthesized by 20th passage cultures of fibroblasts from diabetics and controls.

α and β chains from purified human fetal skin collagen (6) were run on gels under identical conditions and stained with Coomasie Blue. Goldberg and coworkers, using this procedure, identified peaks believed to represent procollagen, a pro α 1 dimer, pro α 1, pro α 2, altered pro α 1 and pro α 2, and α 1 and α 2 chains (4, 5).

RESULTS: More protein was synthesized by diabetic than control cells. Confluent cultures appeared by inspection to have similar cell densities at the time of labeling. However, the increased synthesis in cultures of cells from diabetics was apparent in three different experiments (Table 1).

Interpretations of acrylamide gel peaks are based on analyses by Goldberg and coworkers (4, 5). Gel runs of media collagen are shown in Figure 1. The large peak at 6-12 mm in controls probably represents procollagen. This peak is lower in the case of material synthesized in cultures of diabetic cells

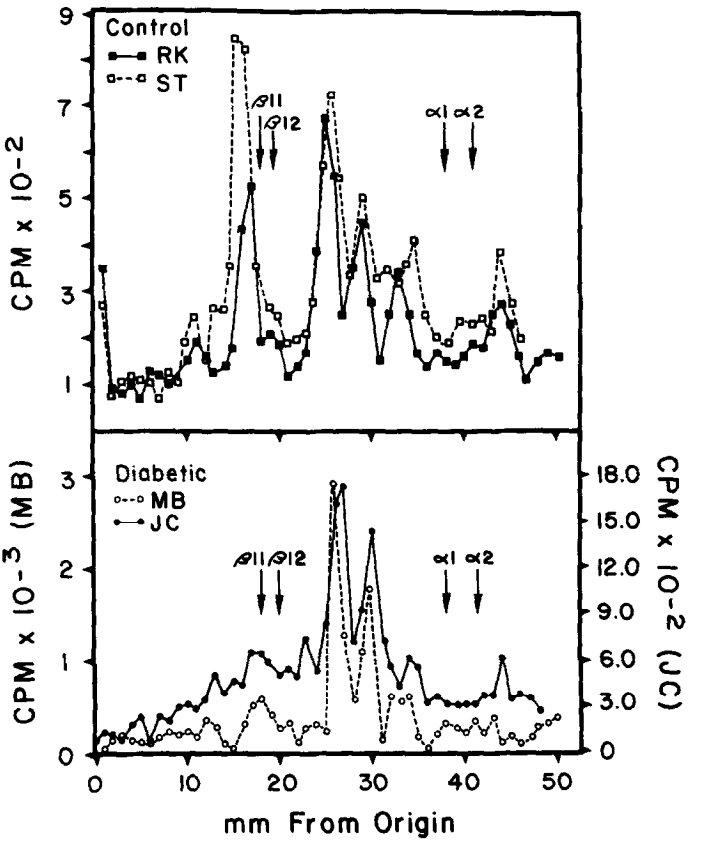

Fig. 2. Acrylamide gel electrophoresis of reduced and alkylated proteins synthesized by 20th passage cultures of fibroblasts from diabetics and controls.

and the latter, in contrast to control cultures, contain large amounts of high molecular weight material (arrow) that remain at the origin. Gel patterns of the reduced and alkylated medium proteins are shown in Figure 2. Peaks between α and β chain markers represent pro α chains and altered pro α chains. The large peak proximal to the β chain markers in the controls was not described in reduced material (4) but is in the location of the pro α 1 dimer seen in non-reduced samples, and probably does represent a dimer. There is a conspicuous absence of this peak in material from cultures of diabetic cells. When media proteins were treated with pepsin, α 1 and α 2

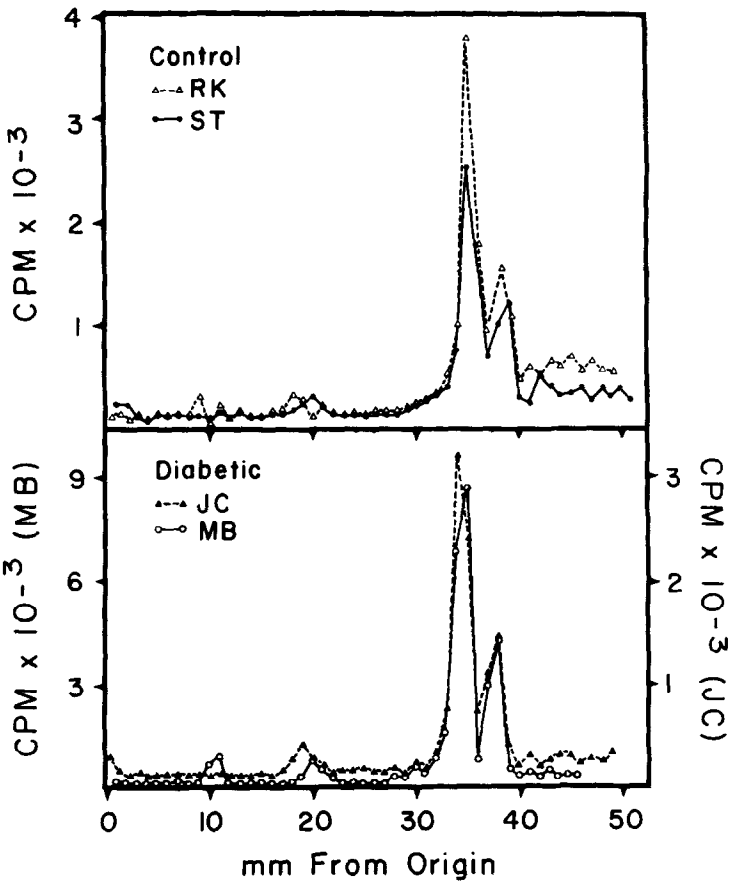

Fig. 3. Acrylamide gel electrophoresis of pepsin-digested proteins synthesized by 20th passage cultures of fibroblasts from diabetics and controls.

chains in the expected 2:1 ratio were obtained. No differences between control and diabetic cultures were apparent (Figure 3).

DISCUSSION: One interpretation of the results is that there is a cross link present in collagen synthesized by diabetic cells, but not in that synthesized by control cells, that causes the high molecular weight aggregate seen in Figure 1. The bond would have to be cleaved by reduction because the material is not seen in Figure 2. On the other hand, control material would contain a non-

reducible cross link responsible for the presumed dimer seen in Figure 2.; this cross link would be absent in diabetic material. The similarity in α chain patterns of control and diabetic material seen in Figure 3 suggest that differences are in the non-helical peptides that are removed by pepsin.

Extensions of these studies have, in preliminary experiments, shown that there is no significant difference between 16-, 19-, 29-, and 51-year-old controls in gel patterns of collagen precursors, and that the presumed dimer present in controls and absent in diabetics contains label from [^{35}S]cystine.

Any abnormality in collagen synthesis by diabetic fibroblasts could be secondary to selection of a cell line from an original heterogeneous population, or to a generalized alteration in cell behavior, it having been observed in this study that diabetic cells synthesize more protein, and in previous studies that cells from diabetics or offspring of diabetics have decreased plating efficiency (7) and a decreased doubling capacity in vitro (8). A specific abnormality in collagen metabolism could arise anywhere between transcription of procollagen and enzymes involved in collagen metabolism, and transport and extracellular processing of collagen.

The preliminary observations reported here indicate the need for additional studies on collagen metabolism by cells from diabetic individuals, and for investigations of the possibility that an abnormal collagen synthesized in diabetes plays a role in the characteristic complications of diabetes that involve collagen-rich tissues.

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