Collagen metabolism in fibrotic liver

Effects of concanavalin A and aggregated myeloma immunoglobulin G

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We previously have shown [Takahashi & Kobayashi (1982) *Hepatology* 2, 249–254] that the administration of concanavalin A to mice with schistosomiasis caused liver collagen content to be reduced by 50%. Here we report the effects of concanavalin A and aggregated mouse myeloma IgG on liver lysyl oxidase activity and present further evidence concerning the possible mechanism by which the liver collagen content was decreased in infected-treated mice. The lysyl oxidase activity at 8 weeks after infection in both treated mice and untreated infected controls was about 28-fold greater than in the age-matched uninfected controls. The specific radioactivity of intracellular free [¹⁴C]proline, the rate of collagen synthesis, the ratio of collagenase-sensitive, protein-bound, hydroxyproline to proline of collagen and the intracellular degradation of newly synthesized collagen were similar in treated animals and in untreated infected controls. In contrast, the extracellular degradation of newly secreted collagen and the specific radioactivity of protein-bound [¹⁴C]hydroxyproline in the agent-treated groups were about 2-fold greater than those in the untreated infected controls. These results suggest that the observed 50% decrease in content of liver collagen of mice treated with the agents apparently was due to the increased extracellular degradation of newly secreted collagen.

INTRODUCTION

The collagen content of a tissue is determined by a balance between processes of synthesis and degradation (Harkness *et al.*, 1954). Newly secreted collagen molecules may be degraded by the priming action of tissue collagenases (EC 3.4.24.7) (Gross & Lapiere, 1962). The introduction of small amounts of bifunctional cross-links into extracellular collagen may increase resistance to collagenolysis and promote formation of stable fibrils (Vater *et al.*, 1979).

The pathophysiology of collagen metabolism in fibrotic processes is still largely unknown. We have previously shown that fibrotic livers of mice with schistosomiasis exhibit extremely low collagenase activity and actual amount relative to the rate of collagen synthesis and collagen content (Takahashi & Koda, 1984). We suggested that this may be the cause of accumulation of collagen (Takahashi *et al.*, 1980). In other kinds of hepatic fibrosis, the activity of lysyl oxidase, a copper-containing amine oxidase that initiates the formation of lysine-derived cross-links (Pinnell & Martin, 1968a), is increased (Siegel *et al.*, 1978; Siegel, 1979; Carter *et al.*, 1982), and this may also contribute to the progressive accumulation of collagen.

In hepatic murine schistosomiasis, the maximal granuloma formation occurring at 8 weeks after infection (Andrade & Warren, 1964) results from a

T-lymphocyte-mediated host immune response against soluble egg antigen released by the living schistosome eggs (Boros & Warren, 1970). The T-lymphocytes and their products appear to regulate the size of granulomas formed in response to the eggs (Colley, 1976; Philips & Colley, 1978; Colley, 1981). The response to the soluble egg antigen by infected mice can in fact be suppressed by treatment with Con A. That was shown by a 50% decrease in liver collagen content along with a shift in the peak of collagenase production to 7 weeks from the peak at 8 weeks observed in untreated infected controls; this coincided with the period in which collagen accumulation was maximal (Takahashi & Kobayashi, 1982). However, the effects of Con A on lysyl oxidase activity, the extent of hydroxylation of proline residues of collagen, the specific radioactivities of [¹⁴C]proline or hydroxy[¹⁴C]proline of the collagen, and intracellular or extracellular degradation of newly synthesized or secreted collagen in fibrotic livers have not been studied. Recently, simple and sensitive procedures for measurement of various parameters of metabolism of collagen in the tissues have been developed by us. Employing these procedures we quantified the effects of Con A and of aggregated myeloma IgG, known stimulants for the production of mononuclear cell factor (interleukin I) (Dayer *et al.*, 1980), on liver lysyl oxidase, and studied the possible pathways by which they are able to decrease liver collagen content of mice with schistosomiasis.

Abbreviations used: Con A, concanavalin A; myeloma IgG, aggregated mouse myeloma IgG; BAPN, β -aminopropionitrile fumarate; dansyl, 5-dimethylaminonaphthalene-1-sulphonyl; GABA, γ -aminobutyric acid; PBS, phosphate-buffered saline (1.5 mM-KH₂PO₄/2.7 mM-KCl/137 mM-NaCl, pH 7.0).

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MATERIALS AND METHODS

Materials

Reagents were obtained as follows: myeloma IgG and mouse IgG from Miles Biochemicals (Elkhart, IN, U.S.A.); diaminopentane dihydrochloride, homovanillic acid, type II horseradish peroxidase (190 units·mg⁻¹) and BAPN from Sigma Chemical Co. (St. Louis, MO, U.S.A.); 4',6-diamino-2-phenylindole dihydrochloride from Boehringer Mannheim G.m.b.H. (Germany); ultra pure urea and guanidinium chloride from Schwarz-Mann (Spring Valley, NY, U.S.A.); DL-6-[³H]lysine (Ci/mmol), L-[U-¹⁴C]proline (237 mCi/mmol), L-[3,4,5(n)-³H]leucine (110 Ci/mol), and ³H₂O (0.25 mCi/g) from New England Nuclear Co. (Boston, MA, U.S.A.); Nonidet P40 from Particle Data Laboratories (Elkhurst, IL, U.S.A.); organ culture medium from Gibco (Grand Island, NY, U.S.A.). Fertile eggs containing embryonic chicks were obtained from Spafas (Norwich, CN, U.S.A.). Female CF-1 mice at 9 weeks of age, each of which had been given a subcutaneous injection of 50 cercariae of a Puerto Rican strain of *Schistosoma mansoni* (Peters & Warren, 1969), were provided by Dr. A. Mahmoud, Case Western Reserve University School of Medicine, Cleveland, OH, U.S.A. Clostridial collagenase was prepared in this laboratory (Takahashi & Seifter, 1972); the specific activity was 1200 units/mg of protein, without any detectable activity against non-collagenous protein substrates. Other reagents and substrates were the same as described in previous publications (Takahashi *et al.*, 1980; Takahashi & Kobayashi, 1982; Takahashi & Koda, 1984; Takahashi & Biempica, 1985).

Administration of reagents

All reagents were sterilized by use of 0.2 µm-pore-size Millipore filters (Millipore Corp., Bedford, MA, U.S.A.). We established the optimum dose of myeloma IgG by injecting infected mice with 10–200 µg of the protein. We found that 60 µg gave the maximal decrease in liver collagen content, with no evidence of animal morbidity. Each group of mice at 5–6 weeks after infection with cercariae was given a daily i.p. (intraperitoneal) injection of Con A (200 µg) or myeloma IgG (60 µg); control group animals were given mouse IgG (60 µg) in 0.1 ml of PBS (Vogt & Dulbecco, 1960). Animals were killed at 8 weeks after infection with cercariae. Seven mice were used in each group studied. Age-matched normal control mice were also given the same dose of each agent.

Preparation of substrate

DL-6-[³H]Lysine-labelled type I collagen substrate was prepared from the calvaria of 17-day-old chick embryos as described by Ham & Tanzer (1979). The SDS/polyacrylamide-gel electrophoretic pattern (Laemmli, 1970) of intact collagen showed a typical type I collagen; this could be digested completely by clostridial collagenase; no solubilized radioactivity was detected when the substrate was allowed to react with trypsin at an enzyme/protein molar ratio of 1:10 at 37 °C for 2–10 h. The specific radioactivity was 1.8×10^6 d.p.m./nmol of collagen.

Preparation of experimental samples

Livers were perfused with PBS as described by Takahashi & Kobayashi (1982); for the study of collagen

synthesis, livers were used without perfusion. For the assays, tissue was homogenized (4 ml/g) in 10 mM-sodium borate, pH 8.2, containing 4 M-urea. The homogenate was centrifuged at 27000 g for 30 min at 4 °C and the supernatant obtained was saved. The residue was re-extracted with the same volume of buffer for 16 h at 4 °C and the extract was combined with the previous one and used for assay. Further extraction with 8 M-urea yielded little lysyl oxidase.

Partial purification of liver lysyl oxidase

Partial purification of liver lysyl oxidase was performed by the procedure of Kuivaniemi *et al.* (1984) and the preparation used for characterization of liver enzyme and for comparative studies of two assay procedures.

Enzyme assay

Before assay, all samples were dialysed against 500 vol. of assay buffer containing 0.1% Nonidet P40 for 24 h. The lysyl oxidase activity was measured by two methods. The first method, developed by Pinnell & Martin (1968b) and modified by Ham & Tanzer (1979), measures the enzymic release of [³H]lysine-labelled collagen substrate in the absence and presence of the inhibitor BAPN (10 µM). Reconstituted collagen substrate (1.8×10^5 d.p.m./0.1 nmol per tube) was used. The recovery controls included a reaction mixture containing no enzyme but known amounts of ³H₂O. The enzyme activity was expressed as the amount of ³H₂O produced/h per mg of DNA or protein.

The second method, developed by Guilbault *et al.* (1967) for assay of amine oxidase, was adapted by Trackman *et al.* (1981) for lysyl oxidase. This procedure involves use of diaminopentane as substrate in the absence or presence of the inhibitor, BAPN, and measures the production of fluorescence by a homovanillate/peroxidase-coupled reaction. The enzyme activity was expressed as the amount of H₂O₂ produced/min per mg of DNA or protein. The assay of partially purified liver lysyl oxidase (0.4–12 ng) by this method was compared with that performed by the first method, and a correlation coefficient (*r*) of 0.95 was obtained.

Determination of collagen content

The collagen content of a tissue was determined by measuring the content of dansylhydroxyproline and expressed as µmol (or nmol) of protein-bound hydroxyproline/mg of DNA, as described previously (Takahashi & Biempica, 1985), with a slight modification. In the present study, dansylhydroxyproline was separated on an octadecyl(C₁₈) column by h.p.l.c. with a modified buffer system based on that of Kaneda *et al.* (1982), using a linear gradient of solvent A (0.01 M-Tris/HCl/methanol, 4:1, v/v, pH 7.75) and 33% solvent B (0.01 M-Tris/HCl/methanol, 1:9, v/v, pH 7.75).

Metabolic labelling

All solutions were sterilized by use of 0.2 µm-pore-size Millipore filters. The labelled proline mixture was purified before use (Berg *et al.*, 1980). The conversion of [¹⁴C]proline into protein-bound hydroxy[¹⁴C]proline was linear with time (1–24 h) and the amount of [¹⁴C]proline (2.70×10^6 – 11.0×10^6 d.p.m.) in the medium. Labelling and pulse-chase experiments were performed at 37 °C in an atmosphere of air/CO₂ (19:1) using tissue explants in Dulbecco–Vogt-modified Eagle's medium (Dulbecco &

Freeman, 1959) supplemented with sodium ascorbate (50 $\mu\text{g}/\text{ml}$) and garamycin sulphate (50 $\mu\text{g}/\text{ml}$). Tissue (80 $\pm$ 20 mg) was placed in 33 mm-diameter culture dishes containing 1 ml of the medium, preincubated for 30 min and labelled with a final concentration of 0.1 mmol of proline containing [^{14}C]proline (5.5×10^6 d.p.m.) for 1 h. As a control the incorporation of [^3H]leucine (5.5×10^4 d.p.m./nmol) into non-collagenous protein was also included. Some dishes were used for the determination of the specific radioactivity of intracellular free [^{14}C]proline. Other dishes were used for the determination of collagen synthesis, secretion and degradation, and still others were used for the determination of the specific radioactivity of hydroxy-[^{14}C]proline or [^{14}C]proline or the ratio of hydroxy-[^{14}C]proline to [^{14}C]proline of the collagen. In some cases, several dishes were pooled to obtain sufficient amounts of material for analysis.

(1) Specific radioactivity of intracellular [^{14}C]proline. This was determined and expressed as d.p.m./nmol. At the end of the labelling period, tissue was washed with medium containing 4 mmol of unlabelled proline (chase medium), rinsed with PBS and, after addition of proteinase inhibitors (0.5 mM-phenylmethanesulphonyl fluoride/10 mM-*N*-ethylmaleimide/10 mM-EDTA), and GABA and norleucine (recovery standards), sonicated in 75% ethanol and centrifuged at 27000 *g* for 30 min at 4 °C. The precipitate was washed with ethanol. The ethanol-soluble fraction and wash solutions were pooled and freeze-dried. The material was then dissolved and chromatographed on a calibrated Sephadex G-25 column (1.0 cm $\times$ 100 cm), with 10 mM-acetic acid. Fractions containing [^{14}C]proline plus GABA were pooled and freeze-dried. The material was then dissolved in PBS, dansylated and contents measured by h.p.l.c. The total amount of [^{14}C]proline (d.p.m.) in the peak was divided by the amount of proline in the same peak.

(2) Rate of collagen synthesis. This was determined by measuring the sum of hydroxy[^{14}C]proline in the 75%-(v/v)-ethanol precipitate and supernatant, and expressed as protein-bound hydroxy[^{14}C]proline synthesized/h per mg of DNA. At the end of the labelling period, proteinase inhibitors were added to the dishes containing newly synthesized and secreted collagen in the tissue and medium, and contents were sonicated together in 75% ethanol. The sonicated material was centrifuged as described above. The precipitate was washed with 75% ethanol. The supernatant and wash solutions were pooled and freeze-dried. GABA was added to both precipitate and dried sample; these were then hydrolysed, dansylated, and the content of hydroxy[^{14}C]proline in the peak measured by h.p.l.c.

(3) Proportion of secreted collagen in the medium. This was determined by measuring the amounts of protein-bound hydroxy[^{14}C]proline in the medium relative to the total newly synthesized collagens (medium + tissue) measured as described above, and expressed as a percentage of synthesized collagen secreted. At the end of the labelling period, labelled tissue was washed with chase medium and incubated in chase medium. After a 3 h chase, proteinase inhibitors were added to the medium, and the medium was collected. The tissue was washed with PBS. The medium containing secreted

collagen and wash solutions were pooled, a known amount of type I collagen carrier was added, then precipitated in 75% ethanol and collected by centrifugation. GABA was added to the precipitate; these samples were then hydrolysed, dansylated and the contents of hydroxy[^{14}C]proline in the peak measured by h.p.l.c.

(4) Specific radioactivity of hydroxy[^{14}C]proline or [^{14}C]proline of collagen. This was determined by measuring the labelled and unlabelled peptides obtained after collagenase digestion of the tissue. For this, the total amount of hydroxy[^{14}C]proline or [^{14}C]proline in the peak was divided by the amount of unlabelled hydroxyproline or proline in the same peak and expressed as d.p.m./nmol. At the end of the labelling period, proteinase inhibitors were added to the medium, and the tissue and medium were dialysed together and digested with collagenase; GABA was added and the material chromatographed on a Sephadex G-25 column as described above. Fractions containing radioactive peptides and GABA were pooled, hydrolysed, dansylated, and the contents of labelled and unlabelled hydroxyproline and proline measured by h.p.l.c.

Collagen degradation

(1) Intracellular degradation of newly synthesized collagen. This was determined by a simplified form of the method of Bienkowski & Engels (1981). For this purpose, the amount of hydroxy[^{14}C]proline appearing in the 75%-ethanol-soluble fraction containing free amino acids or lower- M_r peptides was measured and expressed as d.p.m. of protein-bound hydroxy[^{14}C]proline degraded/h per mg of DNA. The amount of hydroxy[^{14}C]proline appearing in the 75%-ethanol-soluble fraction was measured as described for the rates of collagen synthesis in subsection (2) above. This intracellular degradation was linear with amount of tissue (30–120 mg), amount of [^{14}C]proline (2.70×10^4 – 11.0×10^4 d.p.m./nmol) in the medium, and with time between 1 and 2 h.

(2) Extracellular degradation of newly secreted collagen. This was estimated by measuring hydroxy[^{14}C]proline appearing in the 75%-ethanol-soluble fraction after 24 h chase medium and expressed as d.p.m. of protein-bound hydroxy[^{14}C]proline degraded/24 h per mg of DNA. At the end of the labelling period the tissue was washed with chase medium and incubated in chase medium. After 24 h, dishes were removed, proteinase inhibitors and GABA were added, and a 75%-ethanol fraction of the medium was prepared and collected by centrifugation; this was freeze-dried. The material was then dissolved, hydrolysed and dansylated, and the amount of hydroxy[^{14}C]proline in the peak measured by h.p.l.c.

Other methods

The amino acid content was determined by the method of Rosen (1957), with leucine as standard. The protein content was measured by the method of Bradford (1978), with bovine serum albumin as standard. The DNA content was determined by the method of Kapuscinski & Skoczylas (1977), with sonicated calf thymus DNA as standard. Amino acid analyses were performed on a Beckman 120 C amino acid analyser, the single-column methodology of Moore (1963) being used. H.p.l.c.

equipment used was the same as previously reported (Takahashi & Biempica, 1985). The significance of the data was tested by analysis of variance to determine significant differences between groups; *P* values of < 0.05 are considered to be significant.

RESULTS AND DISCUSSION

We provide here further evidence concerning the pathway by which liver collagen content is decreased in mice with schistosomiasis treated with certain agents (Takahashi & Kobayashi, 1982). This decrease apparently is due to increased extracellular degradation of newly secreted collagens. Lysyl oxidase activity was unchanged by the agents used. The procedures used as described below have allowed us to demonstrate this significant observation.

Liver lysyl oxidase

Lysyl oxidase has been extracted from a variety of tissues by a solvent containing 4–6 M-urea or detergent (Siegel, 1979). In that study the enzyme activity was measured in the presence of 0.1% Nonidet P40. The release of $^3\text{H}_2\text{O}$ from collagen substrate or the production of H_2O_2 from diaminopentane substrate was slightly increased ($10 \pm 3\%$) in the presence of Nonidet P40, in agreement with the report of Ham & Tanzer (1979). In many respects the catalytic properties of liver lysyl oxidase are similar to those of lysyl oxidase isolated from other tissues (Siegel, 1979; Trackman & Kagan, 1979). The partially purified enzyme released $^3\text{H}_2\text{O}$ at a rate of 4.1×10^4 d.p.m./h per mg of protein from native collagen substrate and produced H_2O_2 at a rate of 1450 units/min per mg of protein in the diaminopentane/

peroxidase-coupled reaction, and the Michaelis constant (K_m) was 4.7×10^{-4} M for diaminopentane substrate.

The liver lysyl oxidase activity of mice at 8 weeks after infection with *Schistosoma mansoni* cercariae was similar for animals treated with Con A or myeloma IgG, and untreated infected controls (Table 1), and was about 28-fold greater than that of the age-matched untreated normal controls ($P < 0.001$). It should be noted that the partially purified liver lysyl oxidase activity can be inhibited (presumably through a carbohydrate reaction) by preincubation of the enzyme with Con A (100 $\mu\text{g/ml}$) before assay or by the presence of a high concentration of Con A (500–1000 $\mu\text{g/ml}$) in the assay solution. However, myeloma IgG (60–250 $\mu\text{g/ml}$) had little effect on liver lysyl oxidase activity. Similar results were obtained from livers of infected mice treated with Con A or myeloma IgG, suggesting that the decreased liver collagen content in treated animals, as compared with that of the untreated infected controls, appears not to be attributable to the decreased lysyl oxidase activity.

Metabolic labelling

Recently an improved set of simplified procedures for the measurement of parameters of collagen metabolism was developed by us and is summarized in the Materials and methods section. By using these procedures, the following results were obtained. The intracellular free proline of treated and untreated infected controls was similar and averaged 33.4 ± 0.4 nmol/mg of DNA (mean $\pm$ S.E.M., $n = 35$). After labelling tissue with proline (5.5×10^4 d.p.m./nmol) at 37°C for 1 h, the specific radioactivity of intracellular free [^{14}C]proline in each group was similar and averaged 1640.0 ± 20.1 d.p.m./nmol (mean $\pm$ S.E.M., $n = 35$). The recovery of standard GABA was $85 \pm 5\%$. The rate of collagen synthesis in treated and untreated infected control groups were all similar. About 5.4×10^3 d.p.m. of hydroxy[^{14}C]proline/h per mg of DNA were obtained when liver slices of untreated infected controls were incubated in the medium containing [^{14}C]proline as described above (Table 2). This was about 29-fold greater than in the untreated normal controls ($P < 0.001$). The ratio of collagenase-sensitive, protein-bound hydroxy[^{14}C]proline to [^{14}C]proline was similar in the four groups, averaging 0.83 ± 0.01 (mean $\pm$ S.E.M., $n = 28$) and remained constant throughout the incubation (3 h). The specific radioactivities of protein-bound hydroxy[^{14}C]proline were about 2-fold greater in treated groups than in the untreated infected controls and averaged 2.91 ± 0.03 d.p.m./nmol (mean $\pm$ S.E.M., $n = 14$) and 1.57 ± 0.01 d.p.m./nmol (mean $\pm$ S.E.M., $n = 14$) respectively ($P < 0.001$). The recovery standard in each group was similar and averaged $76 \pm 4\%$. On the other hand, the collagen contents were 2-fold less (Table 2) in treated groups than in the untreated infected controls ($P < 0.001$); Con A- or myeloma IgG-treated groups did not differ significantly. The synthesis of non-collagenous proteins (d.p.m./h per mg of DNA) was similar among the Con A-treated group (mean 9130), the myeloma IgG-treated group (mean 9030) and the untreated infected controls (mean 9300). The recovery of standards for each group was similar and averaged $85 \pm 7\%$. The intracellular degradation of newly synthesized collagen was similar among the four groups (about $7.4 \pm 3\%$) (Table 2). The extracellular degradation of

Table 1. Liver lysyl oxidase activity

The lysyl oxidase activity was measured in the livers of mice infected with 50 *S. mansoni* cercariae, with or without treatment with mouse IgG, Con A, or myeloma IgG, and in livers of age-matched untreated normal controls; the amount of enzyme activity was determined at 8 weeks after infection. Lysyl oxidase activity was measured by two methods and expressed as nmol of H_2O_2 produced/min per mg of DNA of liver or of d.p.m. of $^3\text{H}_2\text{O}$ released/h per mg of DNA of liver. Values shown are means $\pm$ S.E.M. ($n = 7$) for two independent experiments in duplicate samples. Assay procedures are given in the text.

Treatment of mice	Lysyl oxidase activity/mg of DNA of liver	
	Production of H_2O_2 (nmol/min)	Released $^3\text{H}_2\text{O}$ (d.p.m./h)
Normal untreated controls	Not done	19.1 ± 0.67
Untreated infected controls	2.5 ± 0.03	540.5 ± 2.70
Mouse IgG-treated	2.2 ± 0.03	455.4 ± 2.45
Con A-treated	2.2 ± 0.03	465.9 ± 2.78
Aggregated myeloma IgG-treated	2.2 ± 0.03	447.7 ± 2.91

Table 2. Rates of collagen degradation relative to synthesized and accumulated collagen

The rates of collagen degradation relative to synthesized and accumulated collagen in the livers of mice 8 weeks after infection with 50 *S. mansoni* cercariae, with or without treatment with indicated agents, and in livers of age-matched untreated normal controls, were measured. Values shown are means $\pm$ S.E.M. ($n = 7$) for two independent experiments, in duplicate samples. Assay conditions are given in the text. The rates of collagen synthesis and degradation of collagen in liver slices (50–100 mg) were measured after incubation with [14 C]proline (5.5×10^4 d.p.m./nmol), and expressed as d.p.m. of protein-bound hydroxy-[14 C]proline synthesized or degraded/h or 24 h/mg of DNA of liver; the percentage is given in parentheses. The proportions of collagens secreted into medium after a 3 h chase were similar among groups and were about $75 \pm 5\%$ of synthesized collagens. Collagen contents of liver slices were measured without incubation and expressed as μ mol of protein-bound hydroxyproline/mg of DNA of liver. Abbreviation used: Hyp, Hydroxyproline.

Treatment of mice	Rate of collagen synthesis (d.p.m. of Hyp/h per mg of DNA of liver)	Collagen degradation		
		Intracellular (d.p.m. of Hyp/h per mg of DNA of liver)	Extracellular (d.p.m. of Hyp/24 h per mg of DNA of liver)	Collagen content (μ mol of Hyp/mg of DNA of liver)
Normal untreated controls	188.8 $\pm$ 2.5 (100)	Not done	150.0 $\pm$ 3.0 (79.5)	0.4 $\pm$ 0.01
Untreated infected controls	5426.8 $\pm$ 53.4 (100)	410.5 $\pm$ 4.1 (7.6)	1372.2 $\pm$ 14.5 (25.3)	2.6 $\pm$ 0.09
Mouse IgG-treated	5090.2 $\pm$ 50.1 (100)	385.9 $\pm$ 4.1 (7.6)	1340.0 $\pm$ 13.5 (26.1)	2.3 $\pm$ 0.09
Con A-treated	4917.8 $\pm$ 49.3 (100)	344.8 $\pm$ 3.8 (7.0)	2245.5 $\pm$ 23.6 (46.6)	1.4 $\pm$ 0.05
Aggregated myeloma IgG-treated	5024.5 $\pm$ 50.1 (100)	385.9 $\pm$ 3.3 (7.7)	2319.5 $\pm$ 23.0 (46.2)	1.3 $\pm$ 0.04

newly secreted collagen in Con A- or myeloma IgG-treated groups was about 2-fold greater (Table 2) than that of the untreated infected controls ($P < 0.001$). Con A- and myeloma IgG-treated groups did not differ significantly.

The specific radioactivity of intracellular free [14 C]proline, the rate of collagen synthesis, the ratio of collagenase-sensitive, protein-bound, hydroxyproline to proline of collagen, and the intracellular degradation of newly synthesized collagen and lysyl oxidase activity in treated animals and untreated infected controls were all respectively similar. In contrast, the extracellular degradation of collagen and the specific radioactivities of hydroxy[14 C]proline and [14 C]proline of collagen in treated groups were about 2-fold greater than those of the untreated infected controls. Thus the decreased collagen content in treated animals appears to result from the increased extracellular degradation of newly secreted collagen. These results agree with our previous observation that collagenase activity and collagenase protein are increased in Con A-treated infected animals relative to untreated infected controls (Takahashi & Koda, 1984). It should be emphasized that the increased production of collagenase and extracellular degradation of collagen occurred only with fibrotic livers of animals that had been treated with the effective dose of Con A at 5–6 weeks after infection; a similar result was reported previously (Takahashi & Kobayashi, 1982). These results suggest that the critical effects of the agents used appear to occur at an early developing stage, before the full formation of collagenous extracellular matrix. The agents had no effect, or were less effective, in the later stages of fibrosis (Takahashi & Kobayashi, 1982). Whether an actual increase in collagenase mRNA occurs in this fibrosis, and no alteration in collagen mRNA occurs in agent-treated animals, remains to be established.

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