Weight loss often results from various experimental conditions including scurvy in guinea pigs, where we showed that decreased collagen synthesis was directly related to weight loss, rather than to defective proline hydroxylation (Chojkier, M., Spanheimer, R., and Peterkofsky, B. (1983) J. Clin. Invest. 72, 826–835). In the study described here, this effect was reproduced by acutely fasting normal guinea pigs receiving vitamin C, as determined by measuring collagen and non-collagen protein production in skin biopsy sections. Collagen production (dpm/μg of DNA) decreased soon after initiating fasting and by 96 h it had reached levels 8–12% of control values. Effects on non-collagen protein were much less severe, so that the percentage of collagen synthesis relative to total protein synthesis was 20–25% of control values after a 96-h fast. These effects were not due to changes in the specific radioactivity of free proline. Refeeding reversed the effects on non-collagen protein production within 24 h, but collagen production did not return to normal until 96 h. The effect of fasting on collagen production was independent of age, sex, ascorbate status, species of animal, and type of connective tissue and also was seen with in vivo labeling. Pulse-chase experiments and analysis of labeled and pre-existing proteins by gel electrophoresis showed no evidence of increased collagen degradation as a result of fasting. Procollagen mRNA was decreased in tissues of fasted animals as determined by cell-free translation and dot-blot hybridization with cDNA probes. In contrast, there was no decrease in translatable mRNAs for non-collagen proteins. These results suggest that loss of nutritional factors other than vitamin C lead to a rapid, specific decrease in collagen synthesis mainly through modulation of mRNA levels.

Collagen is the most abundant protein in vertebrate animals (1) and control of its production and turnover is of major importance. Earlier studies in normal adult animals suggested that collagen was relatively inert (2) but that in young animals the half-life was much shorter (3). Nevertheless, the turnover of collagen relative to other major proteins is slow (4) and, therefore, much of the information about the regulation of collagen metabolism has come from the study of certain disease states where changes in its production occur. Such studies have been carried out using a variety of models including chronic ascorbate (5) and protein deficiency (6), but problems with these models have been found such as marked

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† Recipient of Special Emphasis Research Career Award AM00850.

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nation of radioactive collagen and non-collagen proteins using purified bacterial collagenase (11). Briefly, tissues were minced and homogenized, then proteins in both media and homogenized tissue were precipitated by 10% trichloroacetic acid containing 10 mM proline (final concentrations) except when the free proline pool was analyzed. After 3 washes in 5% trichloroacetic acid, trichloroacetic acid was dissolved in 0.2 M NaOH at 100°C for 5 min, followed by neutralization and collagenase digestion. The collagenase-resistant material was hydrolyzed in 6 N HCl at 150°C for 30 min, cooled, and counted as non-collagen protein. The net production of labeled collagen relative to total labeled proteins is expressed as the relative rate of collagen production as calculated by the formula: collagen dpm × 100/collagen dpm + (non-collagen dpm × 5.4). Multiplication by 5.4 corrects for the abundance of imino acids in collagen (11).

Ascorbic Acid Content in Tissue—Tissues were weighed and then weighed again to determine change in weight. Tissues were homogenized, then proteins in both media and homogenized tissue were precipitated by 10% trichloroacetic acid for 2 h at 4°C. The reaction was stopped with 1 ml of absolute ethanol, and the extracts were pooled and dried under vacuum at room temperature. Samples were assayed by the a,a'-dipyridyl-Fe3+ complex method (12). Specificity for ascorbic acid was assured by pretreatment of samples with or without ascorbic acid oxidase at 25°C for 15 min (13). Ascorbic acid content was calculated from the difference between ascorbic acid oxidase-digested samples and samples without ascorbic acid oxidase.

Degree of Proline Hydroxylation in Newly Synthesized Collagen—The extent of proline hydroxylation was determined as previously described (14) using a dual label method in which the 1H/14C ratio in collagenase digests is compared to the initial 1H/14C ratio of radioactive proline. The degree of hydroxylation is related to differences in these ratios. The fraction of tritium at the 4-tran-positon of the [4-3H]proline had been previously determined to be 0.69 for the isotope batch used in these experiments. Incubations were carried out as described above but with 9 pCi of L-[3,4,5-3H]proline (15.59 Ci/mmol, Amersham) and total RNA. Final concentrations of K+ and Mg2+ were adjusted to 90 mm and 1.25 mM, respectively. Samples were incubated for 2 h at 28°C and RNA was digested by the addition of 20 μl of 1 M Tris-HCl, pH 10, 5 mM l-proline and incubation at 28°C for 30 min. Procollagen and non-collagen protein synthesis was assayed using purified collagenase as described above.

Dot-Blot Hybridization—Escherichia coli clones containing plasmids with procollagen cDNA inserts were kindly provided as follows: chick α1(II), by Young and Sobel (22); mouse α1(I), by Schmidt and de Crombrugghe (23) and rat α1(I), by Rowe (24). Large scale isolation of plasmid DNA after amplification with chloramphenicol was carried out with lysis by SDS and centrifugation to equilibrium in a cesium chloride-ethidium bromide gradient. The cDNA probe was released from the plasmid by incubation with the appropriate restriction enzyme and recovered from low melting temperature agarose (25). The probes were nick-translated with either 100 μCi each of [32P]dATP, [32P]dCTP, [32P]dGTP, and [32P]dTTP (6000 Ci/mmol, New England Nuclear) or 80 μCi each of [35S]dATPαS and [35S]dCTPαS (>1200 Ci/mmol, New England Nuclear) or 80 μCi each of [35S]dATPαS and [35S]dCTPαS (>1200 Ci/mmol, New England Nuclear) (26). The BRL nick translation kit was used and the reaction was stopped by addition of STOP buffer after 1 h at 14°C for 3P and after 3 h at 14°C for 35S. The material was applied to a Sephadex G-100 column (0.5 × 20 cm) and the excluded radioactive peak was collected and pooled.

RESULTS

Collagen Production in Bone of Acutely Fasted and Refed Guinea Pig—The time course of the effect of fasting on protein synthesis in 4-week-old female guinea pigs was determined. Animals were fed ad libitum (control) or fasted for up to 96 h and then refed (fasted). It should be emphasized that all animals received 60 mg of ascorbate daily throughout the experimental period. Parietal bones were labeled in vitro with [3H]proline in the presence of 0.5 μM ascorbate. Table I shows incorporation of label into collagen and non-collagen protein as dpm/μg of DNA (production) in bone of fed and fasted animals. Within 24 h after initiation of the fast, collagen (p < 0.01) and non-collagen protein (p < 0.05) were stained with Coomassie Blue (0.05% in 50% methanol and 10% acetic acid), destained in 7.5% acetic acid and 50% methanol, vacuum-dried, and exposed to Kodak X-Omat film. Tryptic-labeled acid-soluble collagen from chick embryos (11) was used as a standard.

DNA Determination—DNA content was analyzed by the diphenylamine method (18).
Collagen Synthesis in Fasted Animals

Discussion

Collagen Synthesis in Fasted Animals

Time course of collagen and non-collagen protein production in bone during acute fasting and refeeding

Four-week-old guinea pigs were fasted for up to 96 h then refed (Fasted) or fed ad libitum (Control). Throughout the study, all animals received 50 mg per day of ascorbate. At each time point, parietal bones were labeled in vitro with [3H]proline and collagen and non-collagen proteins were determined using purified collagenase as described under "Experimental Procedures." Each point represents the mean of 8 separate incubations from 2 animals. Values from fasted animals were compared to control values for each day and were tested for significance using the unpaired t test (2 tail). In the case of collagen synthesis in the 24-h refed animals, the significance level was greater than anticipated for the percentage difference because of a large S.E.

Table I

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Hours</th>
<th>Collagen</th>
<th></th>
<th>Non-collagen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fasted (F)</td>
<td>Control (C)</td>
<td>(F/C) x 100</td>
<td>Fasted (F)</td>
</tr>
<tr>
<td>Fasted</td>
<td>24</td>
<td>558</td>
<td>1222</td>
<td>46*</td>
<td>3508</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>469</td>
<td>1516</td>
<td>31*</td>
<td>2702</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>259</td>
<td>1199</td>
<td>22*</td>
<td>2914</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>156</td>
<td>1167</td>
<td>13*</td>
<td>2477</td>
</tr>
<tr>
<td>Refed</td>
<td>24</td>
<td>290</td>
<td>1019</td>
<td>28*</td>
<td>3245</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>355</td>
<td>777</td>
<td>46*</td>
<td>2492</td>
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<tr>
<td></td>
<td>72</td>
<td>908</td>
<td>1441</td>
<td>63*</td>
<td>3378</td>
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<tr>
<td></td>
<td>96</td>
<td>908</td>
<td>1296</td>
<td>70</td>
<td>3466</td>
</tr>
</tbody>
</table>

*p < 0.01.

The specificity of the effect of fasting on collagen production can be emphasized by expressing the results as the relative rate of collagen production (percentage of total protein production) which eliminates possible effects on the proline precursor pool or other generalized effects on protein synthesis. The relative rate of collagen production in control bones throughout the experiment averaged 24.1% ± 1.2.

Within 24 h of fasting, the relative rate already was reduced by one-third of control (p < 0.01) and continued to decrease to an average of 6.2% (p < 0.01) at 96 h. Refeeding gradually increased the relative rate until at 96 h it was only slightly less than control value.

The Effect of Fasting on In Vitro Collagen Production Occurs Independently of Age, Sex, and Type of Tissue—Parietal bone from female guinea pigs of different ages showed a similar response to a 96-h fast. The relative rate of collagen production decreased in 1-, 5-, and 12-week-old guinea pigs to 21%, 20%, and 37% of age-matched controls, respectively. There was no significant difference between the effect of fasting on the relative rate in parietal bones from male (31% of control) or female (28% of control) guinea pigs. The same response of the relative rate of collagen production was observed in long bone (40% of control). The effect was also independent of the type of collagen produced by tissues. Fasting caused a similar decrease of the relative rate of collagen production by parietal bone (24% of control) which makes primarily type I collagen, as in cartilage (26% of control), which synthesizes mainly type II collagen.

Relationship of the Decrease in Collagen Production to the Rate of Weight Loss during Acute Fasting—When the relative rate of collagen production measured in vitro was plotted against the per cent change in body weight of the guinea pigs over the 48 h prior to death, a linear relationship was found for both parietal bone (Fig. 1A, r = 0.89) and cartilage (Fig. 1B, r = 0.93). It should be noted that both positive and negative changes in the weight of the animal over the prior 48 h affected the rate of collagen production relative to total protein production as assessed in vitro.

In Vivo Labeling of Connective Tissues—In order to compare the effect of a 96-h fast on collagen production in several tissues, some of which are difficult to label in vitro, acutely fasted and control guinea pigs were labeled with [3H]proline in vivo for 4 h. Tissues were removed and incorporation into collagen and non-collagen protein was measured. In each tissue, there was a significant decrease in collagen production.

FIG. 1. The relative rate of collagen production compared to the percentage of body weight change over the 48 h prior to death. Animals were fed or acutely fasted as described in the legend to Table I. Each point represents the mean of 4 separate in vitro incubations (one animal) of parietal bone (A) and articular cartilage (B) with [3H]proline. Points corresponding to weight gain are from ad libum fed controls. Differences in positive values resulted from natural variation in the growth rates of individual animals. Variations in weight loss were obtained by acutely fasting for varying times (bone) or from normal variation in animal response to a 96-h fast.
in fasted animals compared to controls. In contrast, non-collagen protein production showed tissue variability, with significant decreases occurring only in skin and skeletal muscle. The relative rate of collagen production (Table II) was significantly decreased in all tissues, reflecting the specificity of the effect of fasting on collagen production compared to total protein production.

The relative rates in fasted bone and cartilage calculated as a percentage of the fed values were about one-fourth of normal, which is similar to the decrease seen after in vitro labeling. These results suggest that the specificity of fasting-induced changes in collagen production are not due to in vitro culture techniques and emphasize the generalized effect of fasting on collagen production.

Analysis of Vitamin C in Tissues—Since the guinea pigs were receiving a large excess of vitamin C, it was unlikely that a deficiency of this vitamin could contribute to the effect of fasting on collagen production. Nevertheless, the ascorbate contents of parietal bone and liver from vitamin C-supplemented, fed, and fasted (96 h) guinea pigs were assayed. Bone from fasted animals had 84 nmol/g wet weight compared to 101 nmol/g wet weight in fed animals. Levels of ascorbate from the liver were 250 nmol/g wet weight for fasted and 285 nmol/g wet weight for fed. These levels in fed and fasted tissues were not significantly different and are both an order of magnitude higher than levels found in scorbatic tissue (9).

To assess the functional availability of ascorbate, tissue levels of proline hydroxylation were measured after in vitro incubation of parietal bone and cartilage from ascorbate-supplemented fed and fasted (96 h) guinea pigs. There was no difference in the percentage of proline hydroxylation in collagen from bone or cartilage of fed and fasted animals labeled in vitro (bone: 42% fasted, 36% fed; cartilage: 47% fasted, 43% fed). These values fall within the normal range for types I and II collagen. The addition of ascorbate in vitro did not increase proline hydroxylation in either fed or fasted tissues, indicating that adequate ascorbate levels for maximal proline hydroxylation were present in the tissues upon removal from the animal.

Further evidence that the effect of fasting on collagen production is independent of ascorbate was the observation of a similar effect in the parietal bone of the rat (5-week-old female Sprague Dawley-OM strain), a species which synthesizes its own vitamin C. In response to a 96-h fast, the relative rate of collagen production was significantly reduced (p < 0.01) from 17.1% ± 1.7% in the fed rat to 8.1% ± 0.9% in the fasted rat, a reduction to 47% of the control.

Specific Radioactivity of the Free Proline Pool and the Absolute Rate of Collagen Production—A potential explanation for the difference in the rate of non-collagen protein production between fed and fasted animals could have been that there was a dilution of the specific activity of the free proline pool in fasted tissue. This would not, however, explain the specific effect on the relative rate of collagen production. The specific activity of [3H]proline from in vitro-labeled parietal bone and cartilage from 96-h fasted and control guinea pigs was determined (Table III). In both bone and cartilage, the specific activity was higher in fasted than in fed animals and therefore could not account for the lower rate of incorporation of label into protein in the fasted animals. The specific activity of the free proline pool was used to calculate absolute rates of production for both collagen and non-collagen protein. These paralleled changes in incorporation, with a 90% decrease in collagen production but only a 50% decrease in non-collagen protein production of fasted tissues, resulting in relative rates of collagen production approximately 20–25% of those in control tissues.

TABLE II

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Relative rate of collagen production (F/C)</th>
<th>%</th>
<th>%</th>
<th>(F/C) × 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasted (F)</td>
<td>Control (C)</td>
<td>(F/C)</td>
<td></td>
</tr>
<tr>
<td>Parietal bone</td>
<td>12.0 ± 1.2*</td>
<td>51.5 ± 0.6</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Long bone</td>
<td>6.0 ± 1.8*</td>
<td>23.8 ± 0.9</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Cartilage</td>
<td>4.2 ± 1.1*</td>
<td>18.8 ± 1.5</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>0.5 ± 0.15*</td>
<td>12.8 ± 1.1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.6 ± 0.1*</td>
<td>2.0 ± 0.6</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.17 ± 0.07*</td>
<td>1.6 ± 0.2</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.01.
* p < 0.05.

Further evidence that the effect of fasting on collagen production is independent of ascorbate was the observation of a similar effect in the parietal bone of the rat (5-week-old female Sprague Dawley-OM strain), a species which synthesizes its own vitamin C. In response to a 96-h fast, the relative rate of collagen production was significantly reduced (p < 0.01) from 17.1% ± 1.7% in the fed rat to 8.1% ± 0.9% in the fasted rat, a reduction to 47% of the control.

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TABLE III

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Proline specific activity</th>
<th>Collagen production</th>
<th>Non-collagen production</th>
<th>Collagen relative rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm/nmol</td>
<td>pmol/μg DNA</td>
<td>%</td>
<td>Bone</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cartilage</td>
<td></td>
<td></td>
<td>Fasted</td>
</tr>
<tr>
<td></td>
<td>Fed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Collagen Synthesis in Fasted Animals

- The effect of fasting on the relative rate of collagen production in various tissues measured by in vivo labeling.
- Pulse-Chase Labeling: To determine if in vitro degradation of newly synthesized collagen accounted for the difference in collagen production between fed and fasted animals, excess unlabeled proline was added to the incubation media to a final concentration of 100 μM at 60 min. Some samples were processed immediately while incubation of others was continued.
Collagen Synthesis in Fasted Animals

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Collagen Synthesis in Fasted Animals

Fig. 2. Analysis of pre-existing and newly synthesized collagens. Parietal bone (lanes 1 and 2) and articular cartilage (lanes 3 and 4) from ad libitum fed (FED) and 96-h fasted (FASTED) guinea pigs were labeled in vitro with [14C]proline. Equal weighted samples of lyophilized tissue homogenates were treated with pepsin as described under "Experimental Procedures" and applied to a 7% SDS-polyacrylamide gel. A, Coomassie Blue stain; B, fluorogram of A.

ued up to 120 min. The radioactivity (dpm/μg DNA) in cartilage collagen (Fig. 3) did not change significantly during the 60-min chase period, indicating that there was no significant degradation in tissue from either fed or fasted animals. Cartilage labeled without the chase of unlabeled proline at 60 min incorporated approximately twice as much radioactivity in 2 h as the 60-min sample, demonstrating the linearity of incorporation. Non-collagen protein production also did not change during the chase so that the percentage of collagen in cartilage from fasted and fed animals remained constant. In this experiment, there was no effect of fasting on non-collagen protein production.

In Vitro Translation—In order to determine at what level fasting-induced regulation of collagen production occurred, mRNA concentrations were determined. Total RNA was extracted from bone and cartilage of fed and fasted guinea pigs and was translated in a reticulocyte lysate system. Formation of collagen and non-collagen proteins was measured using purified bacterial collagenase. Results are shown for assays using total RNA from bone (Fig. 4) and cartilage (Fig. 5) from guinea pigs fed ad libitum (FED) or fasted for 96 h (FASTED).

As shown in the upper panel of Fig. 4, translation of bone non-collagen proteins was only slightly less in fasted than fed samples, while the lower panel shows that there was a marked decrease in the synthesis of procollagen peptides to about 33% of the control level. Total RNA isolated from cartilage showed a similar translation pattern (Fig. 5).

Dot-Blot Hybridization—To confirm that the decrease in translation of procollagen from RNA of fasted animals was due to decreased levels of procollagen mRNAs rather than to specific inhibition of their cell-free translation, we used dot-blot hybridization with specific probes for the mRNAs of the pro-α1 and pro-α2 chains of type I procollagen and for the pro-α1(II) chain (Fig. 6). The decreases in hybridization of type I mRNAs of bone were much greater (90%) than the decrease in translatable mRNA (67%), but the decrease in hybridizable pro-α1(II)mRNA was similar to that found by translation.
Collagen Synthesis in Fasted Animals

Figure 4. In vitro translation of parietal bone total RNA. RNA was extracted from ad libitum fed (••••) or 96-h fasted (O---O) guinea pig bone and was translated in a reticulocyte cell-free system. Non-collagen protein (upper) and collagen (lower) syntheses were determined by purified collagenase digestion of the translation products. Each point is the mean of 3 or more incubations ± S.E.

DISCUSSION

The rapidity and the specificity of the effects of fasting on collagen production suggest that its regulation in intact animals is more responsive to changing metabolic conditions than previously suspected. Within 24 h after initiating fasting in guinea pigs, collagen production, as measured in vitro, dropped to less than one-half of the level in fed controls while after 96 h it was 13% of control. In addition, collagen and non-collagen proteins appear to be regulated separately in response to fasting. Collagen production decreased daily with fasting and on refeeding increased daily. Non-collagen protein production decreased by only 23% in 24 h and relatively little after that and recovered within 24 h of refeeding. Relatively few other systems show such rapid and specific regulation of collagen synthesis. In cultured cells transformed with a temperature-sensitive sarcoma virus, collagen synthesis can be increased or decreased within 24 h by switching from a non-permissive to permissive temperature and vice versa (27).

The decreased collagen production in fasting observed in our study appears to be due to a specific decrease in collagen synthesis, rather than to an increased degradation of collagen. Results of the pulse-chase experiments showed no loss of newly synthesized collagen over a 1-h chase in cartilage from fed and fasted animals. While this technique would not detect rapid degradation, results of our previous studies (9) suggest that such degradation does not play a major role in regulation of collagen production in guinea pig tissues. In those studies, we measured the accumulation of radioactive free hydroxyproline as an index of rapid intracellular plus extracellular degradation. The rate of degradation was only about 10% of

Figure 5. In vitro translation of articular cartilage total RNA. The procedures were the same as described in the legend to Fig. 4 except that total RNA was extracted from articular cartilage.

Table 1. Dot-blot hybridization assay for procollagen mRNA levels. Guineas pigs were fasted for 96 h or fed ad libitum as described in the legend to Table I. Total RNA was extracted from tissues, applied to nitrocellulose filters, and hybridized with labeled probes specific for the collagen types indicated, as described under "Experimental Procedures." Fluorograms were scanned and quantitated, and arbitrary units per μg of RNA were calculated for each dot. The means of values in the linear ranges were used to calculate the percentage of hybridization for tissue RNA from fasted animals compared to fed controls. The percentage for pro-α1(I) is the average of two separate hybridizations.

![Diagram and Table](image-url)
the synthetic rate and was not affected during the late stages of scurvy, which is comparable to a chronic fasting state. Our current study also showed that there was no significant degradation of pre-existing collagen matrix in bone or cartilage from acutely fasted guinea pigs compared to controls, when examined by Coomassie Blue staining of SDS/polyacrylamide gels. When the fluorograms of these same gels were examined, there was a marked decrease in newly synthesized (labeled) type I and type II collagens in fasted animals compared to fed controls. Further support for the conclusion that synthesis rather than degradation was affected by fasting were the observations that there was a specific decrease in the translatable and hybridizable procollagen mRNAs from fasted bone and cartilage. The translation assay showed a specific decrease in mRNAs for types I and II procollagen in tissues from 96-h fasted guinea pigs, with no change in the mRNAs for non-collagen proteins. While these results correlate well with the decreases in the relative rate (percentage) of collagen synthesis, they do not reflect the reduced incorporation into non-collagen protein observed in some experiments. This may be due to the fact that the latter effect was variable, with decreases of 20–50% in some experiments (Tables I and III) or no effect in others (Fig. 3). Such variation may have resulted from differences in the response of some animals to acute fasting. Regardless of whether there was such a generalized inhibition of protein production or not, the extent of the fasting-induced decrease in the relative rate of collagen production was fairly constant in bone and cartilage tissues, suggesting that the specific effect on collagen synthesis occurred independently of the generalized effect.

The effect of fasting on collagen synthesis clearly appears to occur independently of ascorbate. In addition to supplementing all guinea pigs throughout the study with a large oral dose of ascorbate, we measured tissue levels of ascorbate and functional availability of ascorbate (proline hydroxylation) and found no significant difference between tissues from fed and 96-h fasted animals. Rats, a species that synthesizes cartilage growth, showed a similar specific decrease in collagen production in response to a 96-h fast. These studies establish that collagen synthesis can respond to changes in nutrients other than ascorbate.

The rapid occurrence of a decrease in collagen production with fasting makes it unlikely that any vitamin deficiency is involved. Fat-soluble vitamins are stored in the liver and adipose tissue at levels sufficient for several months, ruling out involvement of these vitamins (29). With regard to the water-soluble vitamins, folic acid deficiency is the most rapidly developed, with the first sign being a decrease in growth within 10–14 days after complete withdrawal of the vitamin (30). In rats deficient in folic acid for 14 days, the amount of hot trichloroacetic acid-extractable skin collagen was decreased to the same degree to which generalized protein synthesis was decreased. Thus no specific effect on collagen production was found. Both thiamin (31) and vitamin B₆ (32) deficiencies result in decreased neutral salt-extractable skin collagen after 4–5 weeks, but the effects of these vitamin deficiencies on the weight of the animal or on general protein synthesis were not reported, so that the effect on collagen may not be specific.

Deficiency of one or more of the major nutrients is another possible, although unlikely, factor in the decreased collagen production seen in acute fasting. There have been no reports of the effect of feeding carbohydrate-, fat-, or protein-deficient diets for short time periods on specific protein production. In long term studies, animals given free access to low (33) or totally deficient (34) protein diets over several weeks lost 10–50% of their initial body weight and were found to have decreased collagen production in skin. It is not possible, however, to separate the effects of weight loss from the effects of protein deficiency on collagen production in those studies. The linear relationships between changes in weight and the relative rate of collagen production which we observed appear to extend to animals whose weight is stable or even increasing, although at less than the maximal growth rate. Therefore, in any experimental setting, it would be important to match changes in weight that are either positive or negative when measuring collagen production.

The demonstration of a rapid effect of fasting on collagen production in multiple tissues (parietal bone, long bone, cartilage, lung, skeletal muscle, and skin) suggests a circulating regulatory factor. Potential mediators of this response may be certain hormones that respond to fasting in other systems. Circulating insulin levels are nearly undetectable within 24 h of fasting in rat and mouse (35) and insulin at physiologic concentrations has been shown to increase synthesis of type I collagen in fetal rat calvaria incubated in vitro for 96 h (36). The glucocorticoid hormones are increased in fasting and elevated levels of glucocorticoids have been shown to decrease collagen polypeptide synthesis when skin was exposed to glucocorticoid either in vitro (38) or in vivo (37). The mechanism of this glucocorticoid effect is through a selective reduction of translatable procollagen mRNA (38). Growth hormone levels are slightly increased in fasting (39), but the effects of growth hormone on connective tissues is thought to be mediated through the somatomedins. The somatomedins are a class of peptide hormones that stimulate bone and cartilage growth (40) as well as proteoglycan (41) and collagen production (42). In fasting, there are decreased amounts of somatomedins in spite of elevated growth hormone levels (43). It seems possible that one or more of these hormones play a role in the fasting-induced decrease in collagen synthesis which we have observed. Decreased collagen synthesis in scurvy, which appears to be due mainly to the chronic fasting state induced by vitamin C deficiency, probably occurs by the same mechanism.

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