Radioimmunoassay of Human Collagenase

SPECIFICITY OF THE ASSAY AND QUANTITATIVE DETERMINATION OF IN VIVO AND IN VITRO HUMAN SKIN COLLAGENASE

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SUMMARY

A specific, highly sensitive radioimmunoassay has been developed for human skin collagenase. The assay is based upon competition of purified collagenase, or collagenase of tissue extracts, with isotopically labeled purified enzyme for specific antibody binding sites after which a second antibody precipitation is used to separate bound ¹²⁵I-labeled human skin collagenase from free enzyme. The radioimmunoassay provides a range of 2 to 40 ng for determining enzyme concentration and has permitted quantitation of collagenase in crude tissue extracts of human skin under conditions in which enzymatic activity cannot be detected. In addition, other human collagenases can also be measured with 200 to 300 times greater sensitivity than can be achieved by enzymatic techniques.

In comparative studies, the ability of a variety of human and animal collagenases to compete with ¹²⁵I-labeled human skin collagenase for antibody binding sites has been assessed as a measure of the similarity between these enzymes. Human synovial, gingival, and granulocytic collagenases are identical in their cross-reactivity with human skin collagenase in the immunoassay, supporting the concept that extensive homologies probably exist among collagenases from various human organs. Although collagenase from another primate, the baboon, shows marked cross-reactivity with the human enzymes, collagenases from more distantly related species cross-react only at protein concentrations which are 20 to 30 times greater than those needed to detect the human enzymes. These findings suggest that fewer similarities to the human enzymes will be found when purified preparations of these animal collagenases become available.

Although a variety of specific neutral collagenases from both human and animal tissues has been isolated from culture medium in which living tissue explants were grown (see review in Reference 1), it has not been established that the enzyme activity present in the culture medium is an accurate indication of the actual in vivo synthetic activity of the original tissue or merely a reflection of its ability to synthesize collagenase in vitro. A major deterrent to an accurate assessment of the significance of collagenase in the in vivo degradation of collagen has been the inability of currently available techniques to detect collagenase activity in tissue extracts (1). However, it has been possible to demonstrate readily the existence of collagenase in extracts of human and tadpole skin (2) and in rheumatoid synovium (3) using nonspecific antisera against preparations of collagenases purified from the medium in which these tissues were cultured (3, 4). It has been suggested that the failure to detect enzymatic activity directly in tissue extracts in the presence of immunoreactive collagenase may be due, in part, to the presence in the crude extracts of serum antiproteases (2, 3), which are known inhibitors of both human and animal collagenases (5). In addition, recent evidence indicates that collagenases from tadpole (6) and mouse bone (7) are produced as proenzymes and, although a zymogen for human collagenase has not been demonstrated (8), such a possibility cannot be excluded. Thus, in order to determine quantitatively the levels of collagenase in vivo, it seemed necessary to develop a method for measuring collagenase that is independent of enzymatic activity.

This report details the development of a specific, highly sensitive radioimmunoassay for human skin collagenase. The assay can be used to measure collagenase levels quantitatively in biological materials under conditions where enzymatic activity cannot be detected. In addition, the assay can be used to measure collagenase from other human tissues and to compare collagenases from a variety of animal sources with human enzymes by assessing their relative binding capabilities in the radioimmunoassay.

METHODS

Preparation of Collagenases—Human skin collagenase, rheumatoid synovial collagenase and gingival collagenase were obtained from tissue culture using techniques previously described (9-11). Collagenase from human granulocytes was prepared as described by Lazarus et al. (12). Purified bacterial collagenase (Clostridium histolyticum) was purchased from Worthington Biochemical. Tadpole, mouse skin, rat skin, and rat uterus collagenases were harvested from tissue culture medium using established tech-
Electrophoresis, the enzyme was allowed to react with Tris-HCl (pH 7.4). Collagenase preparations or crude tissue extracts of human skin were subjected to electrophoresis at 125 volts for 90 min in the same Tris-HCl buffer. Following electrophoresis, the enzyme was allowed to react with anti-human skin collagenase antisera at 25°C for 48 hours.

Radioiodination of Human Skin Collagenase—Human skin collagenase purified by affinity chromatography was initially iodinated by the method of Greenwood et al. (18). A typical reaction mixture contained 10 to 25 µg of human skin collagenase (20 µl); 1 to 2 µCi of carrier-free Na-125I (10 µl), 100 µg of chloramine-T (25 µl), and 0.2 M Tris-HCl, pH 7.4 (50 µl). After allowing the reaction to proceed for 1 to 2 min at 25°C, it was stopped by the addition of 240 µg of sodium metabisulfite. 125I-HSC was separated from free 125I by gel filtration on a column (0.9 x 16 cm) of Sephadex G-25 previously saturated with 0.15 M NaCl and adjusted to pH 7.4. The supernatant fractions from each reaction mixture were pooled and examined for collagenase activity by incubation on [14C]labeled collagen fibers for 24 hours at 37°C. A typical reaction mixture consisted of 50 µl of 0.4% 14C-labeled collagen, containing 2500 to 4000 cpm, 50 µl of 0.05 M Tris-HCl (pH 7.4) with 0.005 M CaCl2 and 100 µl of the undiluted crude tissue extract. Collagen was purified by the method of Gross (23, 24) and its purity was monitored by the addition of 10 µg of trypsin to a 14C-labeled collagen gel under the same reaction conditions. In every case, this trypsin control represented less than 10% of the total counts. For use in the radioimmunoassay, serial doubling dilutions of the tissue extracts ranging from 1:20 to 1:640 were prepared in 1% buffered bovine serum albumin. Immunoactivity of these crude tissue extracts was stable for at least 2 weeks at -20°C. Recovery was assessed by adding known quantities of purified human skin collagenase to crude tissue extracts and in every case exceeded 95%. Protein was determined by the method of Lowry et al. (25) using a bovine serum albumin standard.

Radioimmunoassay of Human Skin Collagenase—The radioimmunoassay was performed by the double antibody technique (22), using rabbit anti-HSC γ-globulin as the first antibody and goat anti-rabbit γ-globulin in excess as the second antibody.

1 The abbreviation used is: HSC, human skin collagenase.
toward the cathode and give closely corresponding precipitin arcs after reaction with anti-human skin collagenase antibody. No precipitin bands are seen when this antiserum is reacted with normal human serum under identical conditions. Previous studies, using operationally monospecific antiserum to human skin collagenase (4), demonstrated that crude extracts of human skin, when examined by immunodiffusion, contained material immunologically identical with the enzymatically active collagenase isolated from the medium of cultured skin explants (2). Thus, both the electrophoretic behavior and the immunologic reaction pattern indicate that collagenases from in vitro and in vivo sources are closely related and that immunologic techniques can, therefore, be used to measure enzyme protein in skin extracts. In addition, the single precipitin are obtained by reacting anti-HSC antiserum either with highly purified human skin collagenase or with crude collagenase preparations from culture medium or tissue extracts supports the finding that the antiserum is functionally monospecific for the enzyme.

Iodination of Human Skin Collagenase—To achieve the desired sensitivity of the radioimmunoassay, purified human skin collagenase was iodinated with Na $^{125}$I by a modification of the chloramine-T method of Greenwood et al. (18). After subjecting collagenase to this procedure the iodinated enzyme was found to be relatively unstable with a loss of 25 to 30% of its immunopreceptibility after 3 weeks at $-20^\circ$ (see below). In part, this may be related to the lability of the enzyme itself, since highly purified human skin collagenase loses about 75% of its enzyme activity after storage for 2 weeks at $-20^\circ$. Nonetheless, despite the loss of activity of the unlabeled purified collagenase, its immunoreactivity remains constant for up to 2 months when stored under similar conditions.

To determine whether the damage to the enzyme was simply a result of the mild oxidation and reduction by chloramine-T and sodium metabisulfite, respectively, during the iodination procedure, purified collagenase was systematically exposed to these reagents in concentrations used for iodination. Treatment of the enzyme in this fashion produced no detectable change in a standard precipitin reaction.

A number of findings suggest, however, that once iodinated, human skin collagenase undergoes progressive radiation damage from $^{125}$I. At the time of iodination 70 to 80% of the radioactivity in the $^{125}$I-HSC preparation is precipitable with 10% trichloroacetic acid. After 2 to 3 weeks at $-20^\circ$, trichloroacetic acid-precipitable material decreases to 50 to 60%. In addition, comparison of the amount of $^{125}$I-HSC specifically bound by antibody immediately after iodination and after 1 week at $-20^\circ$ shows a decrease of approximately 5% of antibody-bound material. Finally, addition of the protease inhibitor, Trasylol, a substance known to protect both labeled and unlabeled glucagon during incubation in that radioimmunoassay (26) has no effect on the collagenase immunoassay.

Further evidence for an adverse effect of radiation on the enzyme was demonstrated by subjecting $^{125}$I-HSC to gel filtration on Sephadex G-150 at varying lengths of time following iodination. This procedure allows separation of $^{125}$I-HSC having unaltered immunologic properties from $^{125}$I-labeled material showing altered immunoreactivity as a result of radiation damage. Fig. 2 shows the elution patterns of an equal quantity of $^{125}$I-HSC applied to Sephadex G-150 at 4 days (Fig. 2A) and 24 days (Fig. 2B) following iodination. In each case, Peak I contains all of the material specifically bound by anti-HSC γ-globulin. Four days after iodination, $^{125}$I-HSC specifically bound by antiserum (Peak I) represents about 75% of the total counts in the preparation (Fig. 2A). This immunoprecipitable material decreases to 55% at 13 days and to 42% at 24 days (Fig. 2B) after iodination. The steady decrease in Peak I is reflected by an increase in $^{125}$I-containing peptides of smaller molecular weight in Peak II.

In an effort to improve the initial immunoprecipitability of the $^{125}$I-labeled enzyme, collagenase was iodinated at a higher pH (pH 8.3 to 8.5). The rationale for performing the iodination procedure at an alkaline pH is based on the observations of Gandolfi et al. (19) that this pH favors the formation of moniodinated angiotensin which, in contrast to the diiodinated derivative obtained at neutral pH, has immunoreactivity identical with that of the native hormone (19). It is of interest that

![Fig. 1. Immunoelectrophoretic comparison of crude and purified human skin collagenase from culture medium with immuno- reactive collagenase from skin extracts.](image-url)
use of this same procedure for iodination of human skin collagenase results in greater precipitability of $^{125}$I-HSC by anti-HSC $\gamma$-globulin. These findings suggest that, as with angiotensin, tyrosine residues may be involved in the immunoreactivity of human skin collagenase and that conditions favoring the formation of the diiodinated derivative alter the immunoreactivity. It should be emphasized that even with this modification of the iodination conditions, it is still necessary to carry out gel filtration of $^{35}$I-HSC on Sephadex G-150 no longer than 4 days prior to use.

Titration of Anti-HSC $\gamma$-Globulin—Following iodination, $^{125}$I-HSC was reacted with 100-$\mu$l aliquots of successive dilutions of anti-HSC $\gamma$-globulin (undiluted = 25 mg of protein per ml) to determine the optimum concentration of antisemum (Fig. 3). At low dilutions of anti-HSC, maximum binding of $^{125}$I-HSC is 70 to 80%. With subsequent antibody dilutions binding decreases sharply to a plateau of about 40% at anti-HSC dilutions of 1:1000 to 1:3000. Fig. 3 also demonstrates that the addition of 50 ng of unlabeled human skin collagenase causes significant displacement of bound $^{125}$I-HSC at dilutions of antibody greater than 1:500. In almost every instance, 100-$\mu$l of a 1:2500 dilution of $\gamma$-globulin (1.0 $\mu$g of anti-HSC per tube) were chosen for routine use in the immunassay.

Standard Curve for Quantitation of Human Skin Collagenase—Addition of increasing amounts of purified unlabeled human skin collagenase to tubes containing constant amounts of $^{125}$I-HSC and anti-HSC in the standard assay results in a progressive decrease in the amount of radioactivity found in the precipitate. As shown in Fig. 4A, there is a linear portion of the curve in which the amount of antibody-bound $^{125}$I-HSC is proportional to the logarithm of the concentration of unlabeled human skin collagenase added to each tube. This region of the standard curve provides a working range of approximately 2 to 40 ng for determining enzyme concentration. Fig. 4B shows a standard inhibition curve obtained employing serial dilutions of three different crude tissue extracts of human skin. The slopes of these curves are parallel with the standard curve of the purified enzyme, indicating that identical antigenic determinants are being measured. Thus, levels of immunoreactive collagenase in tissue extracts can be expressed in terms of known quantities of the purified enzyme. In practice, results are considered valid only when the values obtained from at least three different dilutions of the tissue extract fall on the linear portion of the standard curve.

$pH$ Optimum—The effect of $pH$ on the binding of $^{125}$I-HSC to antibody was examined over a range of $pH$ 4 to 9 (Fig. 5). At a 1:2000 dilution of anti-HSC $\gamma$-globulin, the $pH$ optimum extends over a broad range between $pH$ 5.5 and $pH$ 7.5 where 40 to 45% of the $^{125}$I-HSC is bound to antibody. Below $pH$ 5.0 and above $pH$ 8.0, $^{35}$I-HSC precipitability decreases sharply. Although maximum binding of labeled enzyme occurs at $pH$ 6.7 to 7.0, Tris-HCl ($pH$ 7.4) buffer was chosen so that collagenase activity of the purified enzyme and of tissue extracts can be assessed under optimum conditions of $pH$ for enzyme activity (9) without significantly compromising immunoreactivity.

Comparative Studies—In order to determine the extent of cross-reactivity of other collagenases with human skin collagenase, serial doubling dilutions (undiluted = 1 ng of protein per ml) of crude preparations of these enzymes were reacted in the radioimmunoassay. Their ability to compete with $^{125}$I-HSC for antibody binding sites was taken as a measure of the similarity between the enzymes.

Fig. 6 shows standard inhibition curves obtained by reacting various dilutions of four human collagenases, skin, synovial, gingival, and granulocyte, in the radioimmunoassay. It is apparent that the slopes of the curves are parallel, thus supporting, by this sensitive technique, the concept that the enzymes are very closely related. In addition, the identity of these enzymes in the radioimmunoassay establishes the validity of measuring not only human skin collagenase by this method but also collagenases from other human sources as well.

Similarly, collagenases from animal sources were evaluated to determine the extent of their cross-reactivity in this system. Binding measurements were carried out by determining the percentage of inhibition of binding by each unlabeled collagenase.
Fig. 5. Effect of pH on binding of 125I-HSC to antibody. Reaction mixtures contained 100 μl of anti-HSC γ-globulin at a 1:2000 dilution and 125I-HSC (11,500 cpm) in a total volume of 250 μl. The pH of each mixture was determined following addition of the second antibody. In control tubes, using nonimmune rabbit γ-globulin, 5.9% of the radioactivity was precipitated. Relative to the amount of 125I-HSC bound to anti-HSC in the absence of competing proteins (27). Table I shows that crude preparations of all of the human collagenases inhibit binding of 125I-HSC by antibody approximately 60% at a protein concentration of 0.1 mg per ml. In contrast, collagenases from most animal sources inhibit binding by 5 to 10% (Table I). It is of interest that collagenase from the only other primate source, baboon gingiva, cross-reacts strongly with the human enzymes. A standard inhibition curve obtained by using serial dilutions of this enzyme, however, is not parallel with the curve of the human enzymes, indicating that the cross-reactivity is not one of complete immunologic identity (Table I, Fig. 7). Comparison of rat skin collagenase by the same technique shows that, while this enzyme is capable of significant displacement of 125I-HSC in the radioimmunoassay, cross-reactivity occurs only at enzyme concentrations which are 20 to 30 times higher than those used to measure human collagenases (Fig. 7). These data make it likely that extensive homology to human collagenases will be found in collagenases from other primate sources, while fewer, but significant, similarities may be found in collagenases from more distantly related species when highly purified preparations of these enzymes are available for sequence analysis (28).

Sensitivity of Radioimmunoassay—Lysis of 14C-labeled native collagen fibrils at 37°C (13) has been used widely to quantitate collagenase activity. Table II compares the sensitivity of the radioactive collagen fibril assay with that achieved by radioimmunoassay. Using human skin, rheumatoid synovial, and gingival collagenases the minimal amount of enzyme protein detectable by enzymatic techniques is about 20 μg. The data in Table II indicate that the radioimmunoassay, however, is 200 to 300 times more sensitive in assessing enzyme protein in the same preparations.

Quantitation of Collagenase in Tissue Extracts—The supernatant fraction of each crude tissue extract of human skin was examined for collagenase activity by incubation of a 100-μl aliquot of the extract with 14C-labeled native collagen fibrils, containing 2800 cpm per substrate gel, for 18 hours at 37°C. In no case was significant enzyme activity demonstrable by this technique. A mean value of 1.1% lysis of the labeled substrate (range = 0 to 5.5%), was obtained from the 53 tissue extracts examined, while the mean value for controls obtained by reacting 10 μg of trypsin with the radioactive collagen substrate gel under the same conditions was 2.7% lysis.

By radioimmunoassay, however, collagenase can be readily quantitated in supernatant fractions of crude extracts of human skin which contain no measurable enzymatic activity. The values for trunk skin of healthy adult males and females (31 and 25 subjects, respectively) are shown in Table III. Whether the data are expressed either as micrograms of collagenase per wet weight or per dry weight, no significant difference (p > 0.05) is seen between the collagenase content of normal skin of males and females. Variation in enzyme concentration obtained from multiple dilutions of the same extracts in the immunoassay was less than 8.0%. These data represent quantitation of in vivo collagenase in healthy adults only. The effects of age, body...
Comparison of effects on human and animal collagenases on immunoprecipitation of $^{125}$I-HSC

Aliquots of 100 μl of the unlabeled collagenase preparations (0.1 mg per ml) were reacted with $^{125}$I-HSC (9140 cpm) and anti-HSC γ-globulin (1:2200) as described under “Methods.” The values reported are the percentages by which binding of $^{125}$I-HSC to antibody is inhibited by unlabeled collagenase. In the absence of competing unlabeled collagenase, 41% of the total labeled human skin collagenase was bound by anti-HSC. Control precipitates in which nonimmune rabbit γ-globulin was used contained 8.0% of the radioactivity.

Unlabeled collagenase Inhibition of binding %

<table>
<thead>
<tr>
<th>Human</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>51.0</td>
</tr>
<tr>
<td>Synovium</td>
<td>58.2</td>
</tr>
<tr>
<td>Gingiva</td>
<td>51.2</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>60.0</td>
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</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium histolyticum</td>
<td>10.3</td>
</tr>
<tr>
<td>Tadpole tailfin</td>
<td>6.7</td>
</tr>
<tr>
<td>Mouse skin</td>
<td>8.3</td>
</tr>
<tr>
<td>Rat skin</td>
<td>8.6</td>
</tr>
<tr>
<td>Rat uterus</td>
<td>5.6</td>
</tr>
<tr>
<td>Baboon gingiva</td>
<td>49.6</td>
</tr>
</tbody>
</table>

For measuring collagenase activity reaction mixtures consisted of 200 μg of [14C]glycine-labeled collagen fibrils, containing 2400 cpm, as a substrate gel and 25 to 30 μg of crude collagenase in a total volume of 150 μl. Incubation was carried out for 6 hours at 37°C in a shaken water bath. These enzyme concentrations produce the minimum amount of measurable activity under these assay conditions. Buffer blank = 4.2% of the radioactivity.

For use in the radioimmunoassay, collagenase preparations containing the protein concentrations used to detect enzymatic activity were serially diluted. Reaction conditions were the same as for the standard curve (see “Methods”). The indicated dilutions represent the highest dilutions at which immunoreactive collagenase is still detectable.

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Radioimmunoassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein [14C]Collagen solubilized</td>
<td>Dilution</td>
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</table>

<table>
<thead>
<tr>
<th>Collagenase</th>
<th>Radioimmunoassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>28.8</td>
</tr>
<tr>
<td>Synovial</td>
<td>25.0</td>
</tr>
<tr>
<td>Gingival</td>
<td>28.3</td>
</tr>
<tr>
<td>Rat skin</td>
<td>6.7</td>
</tr>
<tr>
<td>Baboon gingiva</td>
<td>5.6</td>
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</tbody>
</table>

Quantitation of collagenase in normal human skin by radioimmunoassay

Aliquots of 100 μl of serially diluted crude tissue extracts were evaluated for collagenase content by radioimmunoassay (see “Methods”).

<table>
<thead>
<tr>
<th>Source</th>
<th>Collagenase content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (31)</td>
<td>4.8 ± 2.2</td>
</tr>
<tr>
<td>Females (25)</td>
<td>4.8 ± 2.0</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation

and pathological states. Studies of human skin and rheumatoid synovial collagenases have shown that enzymatically active forms of these enzymes can be detected in crude tissue extracts by gel filtration, a procedure which partially separates collagenase from the serum antiproteases capable of inhibiting its enzymatic activity (2, 3). Despite this finding, recovery of collagenase from tissue extracts is laborious and not quantitative.

To overcome similar problems with other enzymes, immunologic techniques have been utilized. Shapiro et al. (32) applied radial diffusion in agar for determining the trypsin concentration of intestinal contents, and an immune-displacement method has been developed for quantitating an inactive precursor of proline hydroxylase (33). Although the radioimmunoassay has been a specific and sensitive technique for quantitating peptide hormones in biological material, it has been used only to a limited extent for measuring enzymes (34-36). This is perhaps due to the greater lability of enzymes during iodination procedures, as exemplified in the studies of Kolb and Grodsky (35) in which fructose 1,6-diphosphatase was damaged by exposure to chloramine-T. Furthermore, in the present investigation we have shown that iodinated human skin collagenase has an increased sensitivity to radiation damage. This problem has been overcome by purifying $^{125}$I-HSC by gel filtration shortly before use in a manner similar to that employed in radioimmunoassays of growth hormone (20, 21). In order to preserve binding of
specimens of normal human skin by direct measurement

genealogically more distant, species cross-react less strongly, indicating
similarities to human collagenases (Fig. 7, Table I).

baboon, shows marked similarity to the human enzymes in this

case. Indeed, one of the major assets of an immunological assay
would be the ability to quantitate total collagenases and that antibodies to human skin and synovial

zymogens. If such a proenzyme-enzyme system

occur (6, 32-34, 37-41). Indeed, one of the major assets of an

zymogen must also be considered. The studies of Harper et al. (6) on tadpole collagenase and of

on mouse bone collagenase suggest that these enzymes are present as zymogens. If such a proenzyme-enzyme system

zymogen in the range of 40 to 50% at a 1:2500 antibody dilution,
gel filtration of the material should be performed at intervals not
longer than 3 to 4 days prior to use.

In the present study, the use of a specific radioimmunoassay has facilitated the quantitative determination of collagenase from human skin. This can be done either in crude tissue extracts independent of its enzymatic activity (Table III) or in conjunction with collagenase activity in enzyme preparations obtained from tissue culture medium (Table II). The parallel slopes of purified collagenase and tissue extracts in the radioimmunoassay (Fig. 1) indicate the validity of this approach. In addition, the finding on immunoelectrophoresis of identical mobilities of active enzyme obtained from in vitro preparations and of immunoreactive material from human skin extracts (Fig. 1) supports the concept that collagenases from both sources are closely related. Furthermore, identical cross-reactivity in the radioimmunoassay of human collagenases from synovium, gingiva, and granulocytes with the human skin collagenase (Fig. 6) indicates that all of these enzymes can be quantitated with 200 to 300 times greater sensitivity than can be accomplished by enzymatic assay.

Previous failures to detect collagenase activity in crude tissue extracts of human skin and rheumatoid synovium may, in part, be due to the presence of α₂-macroglobulin and α₁-antitrypsin, which are known inhibitors of collagenase activity (3, 5). Despite their inhibitory capabilities, the presence of these α-globulins in tissue extracts has no apparent effect on the radioimmunoassay at the dilutions employed. The possibility that some of the immunologically detectable collagenase in vivo exists in the form of an inactive precursor (zymogen) must also be considered.

The studies of Harper et al. (6) on tadpole collagenase and of Vaes (i) on mouse bone collagenase suggest that these enzymes are present as zymogens. If such a proenzyme-enzyme system is ultimately shown for human skin collagenase, it is very likely, on the basis of immunological studies of other such systems, that cross-reactivity between the zymogen and active enzyme will occur (6, 32-34, 37-41). Indeed, one of the major assets of an immunological assay would be the ability to quantitate total enzyme protein irrespective of whether the enzyme exists in the presence of inhibitors of its activity or in zymogen form.

Previous studies by Bauer et al. (4) using agar gel diffusion have shown that immunological species specificity exists among collagenases and that antibodies to human skin and synovial collagenases inhibit these human enzymes but not collagenases from other species (3, 4). In the present study the degree of similarity between various collagenases has been evaluated by determining their ability to inhibit binding of ¹²⁵I-HSC by anti-HSC. The results obtained (Fig. 6, Table I) reinforce our previous finding that human collagenases are immunologically closely related. In addition, collagenase from another primate, the baboon, shows marked similarity to the human enzymes in this assay (Fig. 7). In contrast, collagenases from other, phylogenetically more distant, species cross-react less strongly, indicating that enzymes from these animals bear fewer structural similarities to human collagenases (Fig. 7, Table I).

The radioimmunoassay for human skin collagenase is sufficiently sensitive to determine the collagenase content in small specimens of normal human skin by direct measurement of the enzyme. This will permit a more accurate assessment of the role of collagenase in human tissues under normal and pathologic conditions without resorting to in vitro techniques.

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