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Identification of Fibroblasts as a Major Site of Albumin Catabolism in Peripheral Tissues*

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Rat serum albumin has been labeled with dilactitol-¹²⁵I-tyramine, (¹²⁵I-DLT) a radioactive tracer which remains entrapped within lysosomes following cellular uptake and degradation of the carrier protein. Similar kinetics of clearance from the rat circulation were observed for albumin labeled conventionally with ¹²⁵I or ¹²⁵I-DLT-albumin, both proteins having circulating half-lives of ~2.2 days. In contrast, the recovery of whole body radioactivity had half-lives of ~2.2 and 5.1 days, respectively, for the two protein preparations, indicating substantial retention of degradation products derived from catabolism of ¹²⁵I-DLT-albumin. Measurement of total and acid-soluble radioactivity in tissues 2 or 4 days after injection of ¹²⁵I-DLT-albumin revealed that skin and muscle accounted for the largest fraction (50–60%) of degradation products in the body. Fibroblasts were identified by autoradiography as the major cell type containing radioactive degradation products in skin and muscle. Fibroblasts were isolated from skin by collagenase digestion, followed by density gradient centrifugation. The amount of acid-soluble radioactivity recovered in these cells was in excellent agreement with that predicted based on acid precipitation of solubilized whole skin preparations. These studies demonstrate for the first time that fibroblasts are a major cell type involved in the degradation of albumin *in vivo*.

A general aim of research in our laboratory is to characterize the mechanisms regulating the catabolism of plasma proteins. As a first step, we set out to determine the tissue and cellular sites of uptake and degradation of these proteins. Identification of the sites of degradation of proteins with the relatively long circulating half-lives characteristic of plasma proteins relies on the use of radioactive tracers which remain entrapped in cellular lysosomes after the protein carrier has been degraded to diffusible catabolites. In previous work we evaluated the tissue sites of albumin catabolism in normal rats using [³H]raffinose as a residualizing tracer (1). The data from that study indicated that the majority of albumin catabolism took place diffusely throughout the body, primarily in skin and muscle. However, because of both the low specific activity of the ³H isotope and the low efficiency of coupling of the label to protein, it was difficult to carry out autoradiographic studies to identify the cell types involved in albumin degradation.

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In the present study, we have used a newly developed (2) high specific activity iodinated ligand, dilactitol-¹²⁵I-tyramine (¹²⁵I-DLT¹), as the residualizing tracer, and present evidence that the fibroblast is a major cell type involved in the catabolism of albumin in peripheral tissue.

MATERIALS AND METHODS

Bovine serum albumin, NaBH₃CN, glutamine, erythrosin B, DNase I, and metrizamide (2-[3-acetamido-5-N-methylacetamido-2,4,6-triiodobenzamido]-2-deoxy-D-glucose) were purchased from Sigma and Na¹²⁵I from New England Nuclear. Ham's F-10 medium with glutamine, fetal calf serum, penicillin (5000 IU/ml)/streptomycin (5000 µg/ml), and fungizone (250 µg/ml) were obtained from KC Biological, Inc., Lenexa, KS. Complete medium consisted of 0.12% NaHCO₃, 1% each penicillin, streptomycin, fungizone, and glutamine, 10% fetal calf serum, and 20 mM HEPES in Ham's F-10 medium, adjusted to pH 7.4. DNase, 0.4%, was prepared in Ca²⁺- and Mg²⁺-free Hanks' salt solution.

Preparation and Labeling of Albumin—Rat serum albumin (RSA) was purified from fresh rat serum by a series of chromatographic steps: affinity chromatography on Affi-Gel Blue (Bio-Rad), followed by ion-exchange chromatography on Whatman DE 52 (3), and finally, gel exclusion chromatography on Sephacryl S-200 (Pharmacia) eluted with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.7 mM KH₂PO₄). The purity of albumin preparations was evaluated after sodium dodecyl sulfate-polyacrylamide gel electrophoresis under denaturing conditions (4) and was typically >95%, both qualitatively by Coomassie Blue staining and quantitatively by slicing and counting gel lanes containing ¹²⁵I-labeled albumin.

RSA was iodinated directly with Na¹²⁵I using Iodogen (Pierce) as previously described (3), or with the residualizing tracer ¹²⁵I-DLT. The synthesis, iodination, and coupling of this label to protein have been described in detail elsewhere (2). Briefly, the ¹²⁵I-DLT (10 nmol, 0.9 mCi) was treated with galactose oxidase to generate terminal aldehyde groups, and the oxidized ligand then coupled to protein (2 mg, 29 nmol) by reductive amination using NaBH₃CN. Labeled protein was separated from unbound ¹²⁵I-DLT by dialysis against 0.1 M NH₄Cl for 1 h at room temperature, followed by an overnight dialysis at 4 °C against several changes of phosphate-buffered saline. About 70% of added label was attached to protein, giving a final specific activity of ~0.3 µCi/µg, 0.02 Ci/µmol. The final labeled protein preparation was >97% precipitable in 10% trichloroacetic acid. Radioactivity was measured in a Tracor Analytic Model 1190 γ counter.

In Vivo Experiments—Sprague-Dawley rats, male and female, 150–200 g, were fed *ad libitum* and maintained on water containing 0.025% NaI (3). Animals were injected intracardially with radiolabeled proteins, and the kinetics of clearance of protein from the circulation was determined from measurement of radioactivity in plasma aliquots taken at desired time intervals. Clearance of radioactivity from the whole body was determined in a well-type γ counter as described previously (3), and/or by measuring radioactivity recovered in excreta from animals maintained in metabolic cages.

Determination of Total and Acid-soluble Radioactivity in Tissues—Animals were killed by overanesthetizing with ether and perfused

¹ The abbreviations used are: ¹²⁵I-DLT, dilactitol-¹²⁵I-tyramine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; RSA, rat serum albumin.

with iced saline, and tissues were removed. Total recovered radioactivity in various tissues was determined by measuring radioactivity in weighed aliquots and multiplying by total organ weight; total tissue weights for muscle, skin, fat, and bone were calculated as per cent of body weight, based on published values (5, 6). The extent of protein degradation was estimated by determining the amount of radioactivity in tissues soluble in 20% trichloroacetic acid. The procedures for acid precipitation of tissues are essentially those described previously in experiments using [^3H]raffinose-labeled RSA (1), except that samples were counted directly, without the need for decolorizing or scintillation fluid. For soft tissues, precipitations were carried out on aliquots (usually 0.2 ml) of homogenates prepared in 2–3 volumes (w/v) of water; for muscle and skin, precipitations were carried out on NaOH extracts of the tissues (1). In control experiments, mixtures of labeled protein and extracted degradation products were added to tissue extracts from uninjected animals prior to acid precipitation. The recovery of acid-soluble and precipitable radioactivity agreed closely (>90%) with that predicted on the basis of the known distribution of radioactivity in the starting mixture.

Autoradiographic Studies—For autoradiographic studies, 80–120 g animals were injected with 0.3–0.5 mCi of ^{125}I -DLT-RSA. Animals were killed at various times, and tissues were removed as rapidly as possible, cut into 1-mm pieces, and fixed overnight in 2% glutaraldehyde (Ted Pella, Inc., Tostin, CA) in phosphate-buffered saline. The samples were then dehydrated through graded alcohol solutions (70–95%) and embedded in Sorvall JB4 methacrylate according to the manufacturer's directions. Recovery of radioactivity in tissue samples through the fixation and embedding steps was $\geq 90\%$. Tissue blocks were cut into 2–3-micron sections, placed on glass slides, and dipped in Kodak Nuclear Track Emulsion, type NTB2. Autoradiograms developed after 2–3 weeks showed excellent visualization of grains over cells.

Isolation of Cells Containing Radioactive Degradation Products from Rat Skin—Cell isolations were carried out 4 days post-injection of ^{125}I -DLT-RSA. The animal was anesthetized, and an area of abdominal skin $\sim 10 \times 8$ cm was shaved first with fur clippers and then with a straight razor to remove all visible hair. The shaved area was swabbed with 70% ethanol and the rat then killed and perfused as above. A large section ($\sim 5 \times 5$ cm) of shaved skin was then surgically separated from the muscle layer. After this point, all procedures were carried out in a laminar flow hood and in sterile containers. The piece of skin was rinsed in a solution of penicillin, streptomycin, and fungizone and then attached with staples to a 4 \times 4-cm plastic weigh boat with the dermal surface facing down.

The skin attached to the weigh boat was digested by sequential treatments with 2 ml of a collagenase (Worthington) solution. For the 1st h of digestion, the enzyme solution contained 0.4% collagenase in complete medium supplemented with an additional 4% penicillin, streptomycin, and fungizone; for the subsequent half-hour digestions, the enzyme solution contained 0.25% collagenase in the same medium. The collagenase digestion mixture was pipetted underneath the skin into the weigh boat, which was then placed inside a covered Petri dish in a 37 $^{\circ}\text{C}$ shaking water bath (70–80 oscillations/min). At the end of each digestion period, the collagenase extract was removed, and a fresh aliquot of the enzyme solution was added to the weigh boat. Each digest was centrifuged at room temperature for 4–5 min at $150 \times g$ in an IEC Centra 7R centrifuge. The supernatant was removed, and the cell pellet was resuspended in a mixture of 0.8 ml of complete medium and 0.2 ml of DNase solution and washed by recentrifugation. Supernatants and cell pellets were counted for radioactivity; the number of cells was determined using a hemocytometer and cell viability based on exclusion of the dye erythrosin B.

Fractionation of Isolated Cells by Density Gradient Centrifugation—An 8-ml continuous density gradient from 2 to 25% metrizamide in complete medium was prepared in a 1.2 \times 11.2-cm Corex (Corning) centrifuge tube which had been silanized with dichlorodimethylsilane (Sigma). An aliquot, 0.8 ml, of a resuspended cell pellet from a collagenase digest of skin was layered onto the top of the gradient, and the tube centrifuged at room temperature at $700 \times g$ for 10 min in an IEC Centra 7R centrifuge. At the end of the centrifugation, the top 0.8-ml sample volume was removed, and the gradient separated into sequential 1-ml aliquots thereafter. Radioactivity and cell number were determined for each fraction as described above.

RESULTS

Kinetics of Albumin Clearance from the Circulation and Whole Body—To verify that attachment of ^{125}I -DLT did not

affect the normal circulating half-life of RSA, a comparison was made of the kinetics of clearance from the circulation of RSA labeled conventionally with ^{125}I and labeled with ^{125}I -DLT using reductive amination. As shown in the upper part of Fig. 1, the rate of loss of radioactivity from plasma was similar for RSA, irrespective of the labeling procedures. The circulating half-life of RSA, extrapolated from the linear portion of the clearance curves, is about 2.2 days, in agreement with previously reported values (1, 3). The y intercept of the extrapolated clearance curves is at 28%, indicating an approximate 3.5:1 distribution for albumin between extra- and intravascular compartments, again in agreement with typical values. Although the kinetics of clearance from plasma are similar, the kinetics of loss of radioactivity from the whole body are significantly different for albumin labeled either directly with ^{125}I or with the residualizing tracer ^{125}I -DLT. The whole body and plasma half-lives of directly labeled ^{125}I -RSA are the same (Fig. 1) as expected, since radioactive

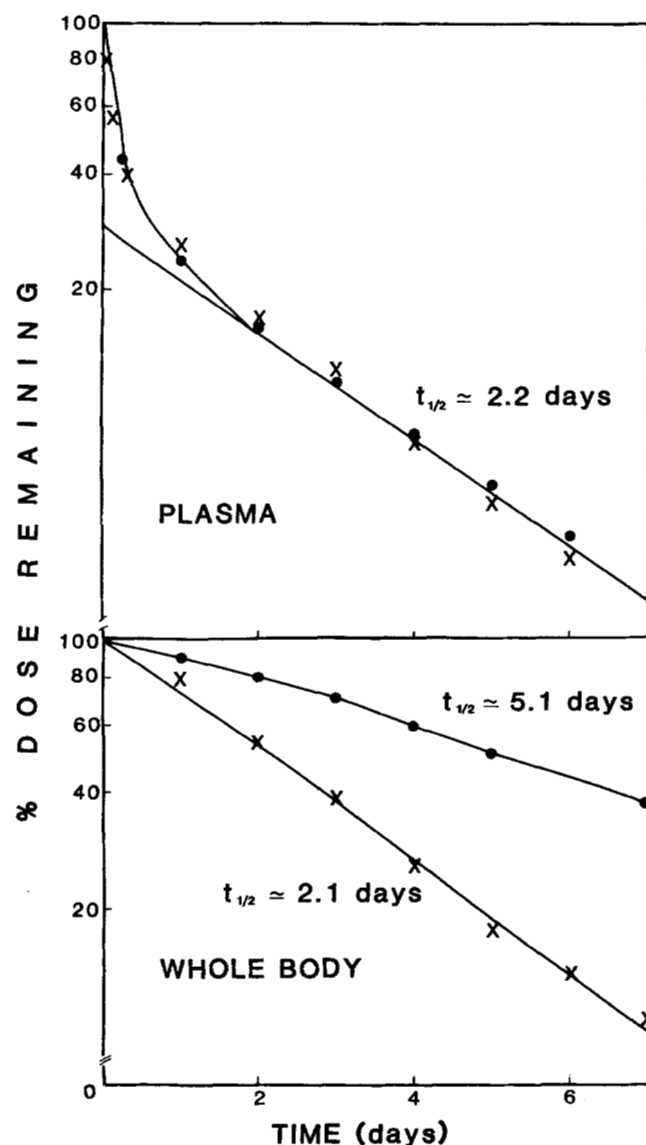


FIG. 1. Kinetics of clearance of various ^{125}I -labeled RSA preparations from the rat circulation and whole body. Animals were injected with $2-3 \times 10^6$ cpm ($\leq 10 \mu\text{g}$) of RSA labeled by direct iodination (x) or with ^{125}I -DLT (•). Whole body radioactivity and radioactivity in plasma were determined as described under "Materials and Methods."

catabolites are rapidly excreted from the body in urine following degradation of ^{125}I -protein (3). In contrast, radioactivity from ^{125}I -DLT-RSA was lost from the whole body with a half-life of about 5.1 days, compared to its circulating half-life of 2.2 days, indicating that substantial amounts of radioactivity from degraded protein were being retained in the body.

Tissue Distribution of Recovered Radioactivity—Table I summarizes the recovery of radioactivity in various body organs at 2 and 4 days after injection of ^{125}I -DLT-RSA. These times were chosen for study because approximately 50 and 70% of injected protein had been catabolized, based on first order kinetics and a half-life of 2.2 days. The data in Column 1 indicate that the majority of radioactivity recovered in the body, exclusive of blood, was in skin and muscle. As shown in Column 2, for skin, muscle, and adipose tissue, the amount of acid-soluble radioactivity (degradation products) increased from 30–50% at 2 days to about 50–70% at 4 days after injection. Skin and muscle accounted for 50–60% of catabolized dose retained in the body (Column 3), similar to results obtained using $[^3\text{H}]$ raffinose-RSA (1). Adipose tissue was not studied previously, but with the greater sensitivity obtained in the present experiments, it is clear that a measurable fraction of albumin catabolism occurred in that tissue.

Total excreted radioactivity accounted for an average of 21 and 41% of administered dose at 2 and 4 days post-injection, respectively. A significant fraction, 20–25% of excreted radioactivity, was recovered in feces, whereas none had been detected in feces using the $[^3\text{H}]$ raffinose label (1). It is possible that the radioactivity in feces may result from protein degradation in hepatocytes, followed by release of catabolites into bile. If the acid-soluble radioactivity in liver and feces is summed, the total amount of albumin degradation in liver may be as much as 10–12%. It is difficult to reach firm conclusions regarding the origin of radioactivity in feces, since

previous studies have shown that a fraction of degradation products arising in non-hepatic tissue can be excreted in feces (2). Since most degradation products released from the body were recovered in urine, it is possible that the small percentage (4%) of catabolites recovered in renal tissue may not result from actual catabolism of albumin in kidney, but rather from resorption of degradation products, as was observed with degradation products from $[^3\text{H}]$ raffinose-labeled proteins (1).

Autoradiographic Studies—A major purpose for using the high specific activity ^{125}I -DLT label was to carry out autoradiographic studies of tissues active in albumin catabolism. Rats were injected with ^{125}I -DLT-RSA and killed 2 or 4 days later. There were no qualitative differences in the distribution of grains in autoradiograms of tissues from the two time points. Photographs of tissues from 4-day animals are shown in Fig. 2, *a–d*, since somewhat more of the radioactivity in skin and muscle is in acid-soluble form, *i.e.* from degraded protein, at this time (Table I).

There were striking concentrations of silver grains over fibroblasts in skin (Fig. 2, *a* and *b*) and muscle (Fig. 2, *c* and *d*), although clearly not every fibroblast was labeled. The identification of these cells as fibroblasts is based on both morphological criteria, *i.e.* their elongated nuclei and thin cytoplasm, and on location in the tissue, *i.e.* in the ground substance of the dermis and in the interstitial region between myocytes in muscle. Besides the concentrations of grains over cells, some label was scattered diffusely in the ground substance in dermis and over myocytes. We interpret the grains over fibroblasts to be derived from degradation products inside these cells, whereas the diffuse background of grains would result from the fraction of acid-precipitable, undegraded protein in extracellular fluid. Although vascular endothelia have been proposed (7) as possible sites of plasma protein degradation, grains were not observed over endothelial cells in any of our sections (see Fig. 2*b* as an example). Thus, either these cells do not play a role in the degradation of albumin, or degradation products were lost from these cells at a significantly greater rate than from fibroblasts. Overall, the autoradiographic studies suggest that fibroblasts are a major site of albumin catabolism in the body. These cells are found diffusely throughout the body, consistent with the broad tissue distribution of degradation products in our experiments (Table I).

Finally, although examined, sections of kidney and liver are not presented since, as discussed above, it is unclear what proportion of the radioactivity in these tissues arises from *in situ* catabolism of albumin, compared to uptake of degradation products released from other tissues. In kidney, there were only sparse numbers of grains associated with glomeruli or in interstitial regions; however, there were relatively heavy concentrations of grains over cells of proximal tubules, suggesting that the cellular radioactivity may indeed result from resorption of degradation products. In liver, silver grains were distributed in a diffuse fashion and not localized over any unique cell type.

Isolation of Fibroblasts Containing ^{125}I -Labeled Degradation Products—Based on the autoradiographic studies, we next attempted to isolate fibroblasts containing radioactive degradation products from the skin of animals injected with ^{125}I -DLT-RSA. Significant yields of intact cells containing radioactivity were obtained routinely by sequential 30-min collagenase digestions of skin, as described under "Materials and Methods." The data in Table II show that ~10% of the total skin radioactivity (Column 2) was released during each digestion period, and an average of ~60% of the released radioactivity was recovered in the washed cell pellet (Column 3). The

TABLE I

Recovery of radioactivity and extent of protein catabolism in tissues following injection of ^{125}I -DLT-RSA in rats

Animals were injected with $\sim 20 \times 10^6$ cpm (90 μg) of ^{125}I -DLT-RSA and killed at 2 or 4 days post-injection. Total and acid-soluble radioactivity in tissues was determined as described under "Materials and Methods."

| Tissue | Time post-injection | | | | | |
|--------------------|--------------------------------------|-----------------|------------------------------|----------------|------------------------------------|------|
| | 2 days | | 4 days | | 4 days | |
| | % cpm recovered in body ^a | | % acid-soluble radioactivity | | % protein catabolized ^b | |
| Blood | 20.9 | 13.6 \pm 1.6 | ≤ 3 | ≤ 3 | | |
| Liver | 7.8 | 8.4 \pm 0.9 | 85.5 | 86.4 \pm 3 | 6.7 | 7.3 |
| Kidney | 4.3 | 4.7 \pm 0.2 | 83 | 88.5 \pm 1 | 3.6 | 4.1 |
| Spleen | 0.5 | 0.7 \pm 0.1 | 71.5 | 85.6 \pm 1.1 | 0.36 | 0.6 |
| Fat | 6.9 | 8.6 \pm 1.3 | 54 | 71.4 \pm 3.6 | 3.7 | 6.2 |
| Bones | 4.4 | 4.9 \pm 0.7 | 53.5 | 64.8 \pm 2.6 | 2.4 | 3.2 |
| Heart | 0.4 | 0.36 \pm 0.02 | 42.5 | 66.2 \pm 3.6 | 0.17 | 0.23 |
| Muscle | 22.3 | 23.3 \pm 1.2 | 32.5 | 53.0 \pm 1.8 | 7.3 | 12.4 |
| Skin | 28.2 | 31.9 \pm 3.5 | 44.3 | 64.6 \pm 2.1 | 12.5 | 20.6 |
| Other ^c | 3.8 | 4.4 \pm 1.1 | ND ^d | ND | | |

^a Data are expressed as per cent of radioactivity recovered in the body, which represented an average of 79 and 59% of administered dose at 2 and 4 days post-injection, respectively (See Fig. 1). The sum of recovered dose in tissues and excreta was $100 \pm 6\%$ of injected dose. Data at 2-day time points are the average values for two animals; data at 4-day time points are for four animals and are expressed as mean \pm S.D.

^b Per cent protein catabolized was determined by multiplying the average per cent recovered dose (Column 1) by the average per cent acid-soluble radioactivity (Column 2).

^c Other: lung, adrenals, thymus, stomach and contents, and intestine and contents.

^d ND = not determined.

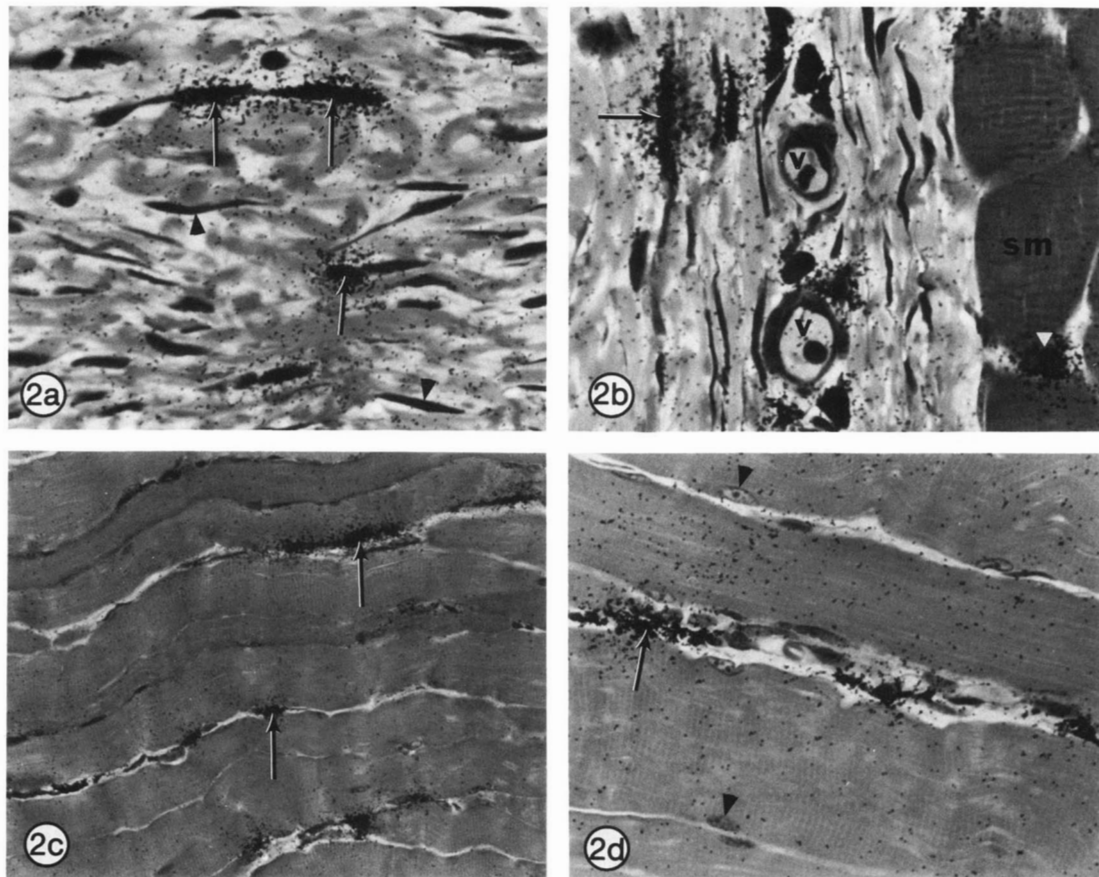


FIG. 2. Autoradiograms of tissues taken 4 days after injection of 300 μ Ci of 125 I-DLT-RSA into an 85-g rat. Tissues were processed for autoradiography as described under "Materials and Methods," and slides developed after 3 weeks. *a*, a typical section of dermis shows the dense concentration of silver grains over some fibroblasts (arrows) while numerous other fibroblasts remain unlabeled (arrow heads). Grains are only diffusely distributed over the extracellular matrix of collagen fibers and ground substance ($\times 230$). *b*, a view of dermis adjacent to skeletal muscle (*sm*). Note the heavy concentration of silver grains over the fibroblasts (arrow) in dermis and muscle (arrow head), but the absence of grains over the endothelium lining the blood vessels (*v*). ($\times 290$). *c* and *d*, low ($\times 290$) and high ($\times 580$) magnifications of skeletal muscle. Many of the fibroblasts located between adjacent myocytes are heavily labeled (arrows). As was seen in skin, not all fibroblasts are labeled. Neither myocyte nuclei (arrow heads in *d*) nor cytoplasm shows accumulation of silver grains.

digestion procedure generally released 50–70% of the total radioactivity in the skin sample during a 3.5-h period. The number of cells in each harvest varied from 1 to 2×10^6 , and the cells were >97% viable based on exclusion of the dye, erythrosin B. As a control for fibroblast lysis in the collagenase extracts, a known number of cultured fibroblasts was added to a digest of skin and incubated for 30 min at 37 °C. In this experiment, $100 \pm 10\%$ of the added cells was recovered, making it likely that the number of cells shown in Table II (Column 4) represent a major fraction of the actual cells in the extracts.

Radioactivity recovered in cell pellets was >95% soluble in 20% trichloroacetic acid, indicating that it represented almost exclusively degradation products entrapped inside cells. In control experiments, when cultured fibroblasts were mixed with the supernatant from a collagenase extract of radioactive skin, <0.02% of the total radioactivity was recovered in the washed cell pellet. Furthermore, when the acid-soluble fraction from either cell pellets or digest supernatants was analyzed for free iodide (8) $\leq 10\%$ of the radioactivity was recovered in the free form. The amount of acid-soluble degradation products recovered in cells is consistent with that predicted on the basis of acid precipitation of whole skin (Table I). Thus, for the experiments in Table II, the acid-

soluble radioactivity in the cell pellets represented 56% of the total radioactivity released, in reasonable agreement with the 65% acid-soluble radioactivity measured in whole skin (Table I). Another 24% of released radioactivity was recovered in acid-soluble form in the digest supernatants. Some of this soluble radioactivity undoubtedly arises from leakage of degradation products from cells, but may also arise from degradation of intact protein during the collagenase digestion. In a control experiment, when 125 I-DLT-RSA was mixed with a digestion extract and incubated for 30 min, about 10% of the radioactivity was converted to acid-soluble form.

In some experiments, the radioactive cell pellets from separate digestions were transferred to individual culture flasks and incubated overnight at 37 °C for recovery of fibroblasts. In these experiments, an average of 27 and 15% of the cells and radioactivity, respectively, seeded in the flasks was recovered after trypsinization of adherent cells on the following day. Many of the cells attached to the culture flasks exhibited typical fibroblast morphology although they were also highly vacuolated, probably because of the HEPES buffer (9) used to maintain pH during the digestion of skin. Since fibroblasts are expected to be the major adhering cell type from cultures of skin cells, the results are qualitatively consistent with the hypothesis, but do not prove, that radioactive degradation

TABLE II

Recovery of radioactivity and cells following sequential collagenase digests of skin from rats injected with ^{125}I -DLT-RSA

Animals ($n = 3$) were injected with $\sim 160 \times 10^6$ cpm of ^{125}I -DLT-RSA and killed after 4 days, and skin was digested with collagenase as described under "Materials and Methods."

| Digest ^a | Total counts/min ^b | Counts/min in cell pellet ^c | Number of cells ($\times 10^{-6}$) | Counts/min/cell ^d |
|---------------------|-------------------------------|--|--------------------------------------|------------------------------|
| | % | % | | |
| 1. | 17.0 ± 3.9 | 51.1 ± 6.6 | $0.99 \pm .28$ | 0.21 |
| 2. | 9.0 ± 2.4 | 61.3 ± 7.3 | $1.52 \pm .45$ | 0.08 |
| 3. | 9.6 ± 3.6 | 40.2 ± 3.8 | $1.62 \pm .2$ | 0.21 |
| 4. | 10.3 ± 1.0 | 66.5 ± 6.8 | $1.94 \pm .13$ | 0.14 |
| 5. | 8.8 ± 1.6 | 64.1 ± 5.4 | $1.35 \pm .1$ | 0.17 |
| 6. | 9.6 ± 3.0 | 77.5 ± 2.5 | $2.14 \pm .24$ | 0.13 |

^a Digests were harvested at 30-min intervals after the 1st h as described under "Material and Methods."

^b Radioactivity in each digest was divided by the sum of radioactivity in all digests and in undigested skin. Total radioactivity in skin samples was $2-4 \times 10^6$ cpm.

^c Cells were separated from the digest by centrifugation, resuspended in fresh medium, and recentrifuged, and the counts/min in the washed cell pellet was divided by the total radioactivity in the original digest.

^d Data shown is for one animal, since the value for counts/min/cell will depend on absolute amount of radioactivity injected and size of the animal. An approximate 2-fold range in counts/min/cell was typical in these experiments.

products are recovered in fibroblasts alone. Efforts to increase the yield of viable cells plated from the collagenase digests have not been fruitful.

As another means to identify the cells containing radioactive degradation products, several different collagenase digests of skin were subjected to centrifugation on a continuous metrizamide density gradient. In control experiments, it was established that normal rat skin fibroblasts isolated from a culture flask by trypsinization banded primarily at fractions 4-6, whereas white cells in peripheral rat blood banded at fractions 6-7 and red blood cells were recovered near the bottom of the gradient in fraction 8. The histograms in Fig. 3 show that the majority of the recovered cells ($\sim 92\%$) and radioactivity ($\sim 81\%$) was associated with fractions 3-5. Although the majority of cultured fibroblasts peaked one fraction below the peak of primary isolates (4 versus 3), we attribute the differences in migration on the gradient primarily to the fact that the freshly isolated cells are highly vacuolated (see above), and thus likely to have a higher water content, making them slightly less dense. Only a limited amount ($<10\%$) of cells or radioactivity from skin was recovered in the region of the gradient associated with blood leukocytes. No overall differences in the gradient profiles were observed if the digest was isolated from an early (digest 2) as compared to a late (digest 5) harvest from skin. There is an indication that there was preferential association of the radioactivity with the lighter fibroblast bands, but the significance of this observation is not yet clear. It can be noted from the autoradiographic studies that not all fibroblasts contain degradation products, and thus the centrifugation technique may be useful for selecting a fraction of cells which is more active in albumin uptake and catabolism.

DISCUSSION

The present studies with ^{125}I -DLT labeled albumin have confirmed our earlier studies with the residualizing label, [^3H] raffinose (1), and identify skin and muscle as primary sites of albumin catabolism in the rat. Although the tissue pattern of albumin degradation is similar in our two studies, there are some differences between conclusions from our work and that

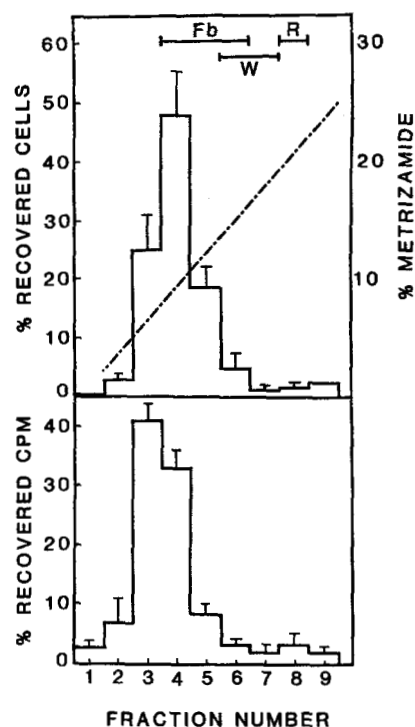


FIG. 3. Distribution of cells and radioactivity after density gradient centrifugation of cells isolated from rat skin. Two digests from each of the three experiments described in Table II were subjected to centrifugation on metrizamide gradients as described under "Materials and Methods." The tops of the blocks represent the means, and the bars the standard deviations for recovered cells and radioactivity from the six gradients. Recovered cells and radioactivity represented 71 ± 17.2 and $72 \pm 7.3\%$, respectively, of the amounts applied. Cultured rat skin fibroblasts (Fb) banded at fractions 4-6, white blood cells (W) from peripheral rat blood at fractions 6-7, and red blood cells (R) at fraction 8.

on albumin catabolism in the rabbit (10), using [^{14}C]sucrose coupled to protein with cyanuric chloride. In the rat, the contribution of liver to total albumin degradation was about 10%, based on the amount of catabolized dose recovered in liver, intestinal contents, and feces, whereas in the rabbit, the value from these same sources totaled about 27%. It is likely, however, that the contribution of the liver to albumin catabolism in the rabbit is overestimated because of hepatic uptake of cyanuric chloride-derived degradation products released from peripheral tissues (2). Another difference between the rat and rabbit studies is in the relative roles of skin and muscle in albumin degradation. Thus, in the rat, skin contributes about twice as much as muscle to overall albumin degradation, whereas the reverse was true in the rabbit (10). Although the source of these discrepancies regarding the quantitative role of the liver and other organs in albumin catabolism is not clear, residualizing labels have proven useful in both the rat and rabbit to establish that albumin is catabolized diffusely, throughout the body, rather than in any specific tissue. In addition, in the rat, catabolites from ^{125}I -DLT-albumin were entrapped sufficiently well inside fibroblasts to allow isolation of these cells containing radioactive degradation products from skin.

It should be noted that in all studies to date (1, 10, this work) a significant fraction of radioactive degradation products from albumin labeled with residualizing tracers is excreted in urine and/or feces. Thus, it is possible that a fraction of albumin catabolism occurs in as yet unidentified tissues or cells from which the degradation products are rapidly released. It is also possible that some albumin catabolism may take

place extracellularly, *e.g.* at the cell surface by membrane-bound proteases, or in extracellular fluid by soluble proteases. Residualizing labels would not identify these sites of albumin degradation, since the degradation products would eventually be excreted through the kidney or liver. With the development of residualizing labels which are entrapped more efficiently in cells, it may be possible not only to identify other cell types active in albumin degradation, but also to assess the significance of albumin catabolism in the extracellular space.

The present studies with ^{125}I -DLT-RSA identify the fibroblast as a major cell type involved in albumin catabolism, both by autoradiographic identification of radioactive fibroblasts in tissues and by density gradient isolation of radioactive fibroblasts from skin. Our results support the hypothesis that the catabolism of albumin in fibroblasts may be physiologically important as a source of amino acids for parenchymal cells in muscle, skin, and other extrahepatic tissues. Thus, the liver, which serves a central role in the synthesis and distribution of carbohydrate and lipid fuels for peripheral tissues, may also secrete albumin as a reservoir of amino acids for use in these tissues. This hypothesis is consistent with the observed net flux of amino acids from extrahepatic tissue toward the liver and the decrease in plasma amino acid concentrations during transit through liver (11). There are also interesting parallels in the regulation of carbohydrate, lipid, and albumin reserves in the body. Thus, the synthesis of glycogen, triglyceride, and albumin (12, 13) is highly sensitive to regulation by insulin, and synthesis of these molecules rapidly decreases in response to protein starvation and

diabetes. In contrast, whereas the catabolism of glycogen and triglycerides is enhanced in starvation and diabetes, the catabolism of albumin is suppressed (3). Further studies are in progress to characterize the mechanisms regulating albumin catabolism in fibroblasts.

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