Glucagon Degradation by Human Mononuclear Cells

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Summary. Degradation of ¹²⁵I-iodoglucagon by human mononuclear cell preparations including one containing 18%–27% monocytes, one consisting of 97% pure monocytes and one consisting of 98% lymphocytes was examined. Intact cells were incubated with ¹²⁵I-iodoglucagon and degradation assessed by measuring an increase in trichloroacetic acid soluble products or in non-immunoprecipitable products. The preparation consisting of intact lymphocytes did not degrade glucagon. Glucagon was degraded by preparations containing monocytes and this degradation increased with time. No difference between monocyte degradation as measured by trichloroacetic acid or immunoprecipitation was found. Degradation by intact monocytes and by mononuclear homogenates increased sixfold from 4°C to 37°C. Subcellular fractionation demonstrated that the majority of the neutral

glucagon degrading activity was in the 100,000 g supernatant (cytosol). Kinetic analyses gave K_m values of 1.1×10^{-5} mol/l, 7.5×10^{-6} mol/l, and 1.2×10^{-5} mol/l for glucagon degradation by intact mononuclear cells, homogenates, and cytosol, respectively. Inhibitor studies indicated a sulphhydryl dependent enzyme was involved in glucagon degradation by both intact cells and cytosol. The monocyte appeared to be the cell responsible for degradation of glucagon by mononuclear cell preparations. The degradation of glucagon under physiological conditions by intact monocytes was mediated by a neutral proteolytic enzyme, primarily localized in the cytosol.

Key words: Glucagon, degradation, monocytes, neutral protease.

The actions of glucagon on sensitive tissues are initiated by binding of the hormone to specific plasma membrane receptors. Activation of the adenylate cyclase system follows the interaction of the hormone-receptor complex giving rise to a cascade of phosphorylation reactions inside the cell which result in the characteristic metabolic response to glucagon [1–3]. Tissues known to be responsive to glucagon include adipocytes and hepatocytes. Recant and co-workers have demonstrated that human mononuclear leucocytes are capable of specifically binding glucagon [4, 5]. Mononuclear cells are not classically considered to be glucagon sensitive and therefore the significance of these receptors is unknown.

The fate of glucagon bound to responsive tissues is not well known although the hormone can be degraded by a variety of cellular and circulating proteases. In this study, the degradation of glucagon by human mononuclear cell preparations was investigated. Ficoll-Hypaque preparations containing 18%–27% monocytes and a 97% pure monocyte preparation were found to degrade ¹²⁵I-iodoglucagon. In contrast, a 98% pure lym-

phocyte preparation possessed negligible glucagon degrading capacity. In addition, glucagon-degrading systems of intact cells and subcellular fractions were examined and partially characterized.

Materials and Methods

Materials

Glucagon was a gift of Dr. R. Chance of Eli Lilly, Indianapolis, Indiana, USA. ¹²⁵I-iodoglucagon was purchased from New England Nuclear Company, Boston, Massachusetts, USA and was uniformly greater than 95% trichloroacetic acid precipitable and 80% immunoprecipitable. When ¹²⁵I-iodoglucagon was more than 2 weeks old, it was rechromatographed on Biogel P-30 (Biorad Laboratories, Richmond, California, USA). Chemicals used in the inhibitor studies were purchased from Sigma Chemicals, St. Louis, Missouri, USA and were of reagent grade quality. All other chemicals were purchased commercially and were of reagent grade quality.

Mononuclear Cell Preparations

Approximately 100 ml of heparinized blood were drawn from fasted normal volunteers and mononuclear cells were isolated using Ficoll-

Fig. 1. Glucagon binding and degradation by varying mononuclear cell concentrations (as per 10^6 cells in 500 μ l), incubated in 0.1 mol/l HEPES-1% bovine serum albumin buffer (pH 7.4) for 30 min at 37 °C. Glucagon degradation was measured by the increase in solubility of 125 I-iodoglucagon in 5% trichloroacetic acid. No glucagon-degrading activity was present in the medium after removal of the cells. $\circ$ = degradation; $\bullet$ = binding

Hypaque [6]. This preparation, which contains both lymphocytes and monocytes, was used for all experiments unless otherwise indicated. To minimize individual differences, each experiment used mononuclear cells freshly isolated from a single subject. The mononuclear cell preparation contained 18%–27% monocytes with the remainder being large lymphocytes. Monocytes were identified by non-specific esterase stain and cell viability was consistently greater than 98% by trypan blue exclusion [7]. For some studies, purified monocytes (97%) were prepared by centrifugal elutriation in the laboratory of Dr. A. Postlewaite and purified lymphocytes (98%) by a gel filtration technique [8].

Glucagon Degradation by Intact Mononuclear Cells

Mononuclear cell preparations, as indicated in figure and table legends, were incubated with 10^{-10} mol/l 125 I-iodoglucagon in 0.1 mol/l HEPES, 1% bovine serum albumin (pH 7.4) in a total volume of 500 μ l at 37 °C in a Dubnoff metabolic shaker (Precision Scientific Products, McGraw Park, Illinois, USA) for 30 min. Aliquots of the incubation mixture were then added to 300 μ l of buffer modified to 2% bovine serum albumin and centrifuged at 17,000 g for 5 min. After removal of the supernatant, the pellet was washed again with 500 μ l of buffer. All supernatants were then combined. Supernatants and pellets were counted in a gamma counter (Packard Instruments, Downers Grove, Illinois, USA) at 58% efficiency. Cell associated radioactivity, including both bound and internalized 125 I-iodoglucagon, was defined as counts per min in the pellet divided by counts per min in the pellet plus supernatant $\times 100$. Degradation was assessed by adding 10% trichloroacetic acid to the supernatant until a final concentration of 5% trichloroacetic acid was obtained. The precipitate was separated by centrifuging the mixture at 2500 rev/min and quantifying precipitated intact hormone and soluble degradation products as previously described [9]. To determine if glucagon-degrading activity was leaking from intact mononuclear cells into the incubation mixture, mononuclear cell fractions were incubated in buffer for 30 min, centrifuged, and the supernatant incubated with 125 I-iodoglucagon as above.

Assay of Degradation by Immunoprecipitation

Preliminary experiments were performed to determine the amount of rabbit anti-glucagon antiserum (kindly provided by Dr. Joseph Ingraham, Indiana University School of Medicine, Indianapolis, Indiana, USA) necessary for complete precipitation of the 125 I-iodoglucagon.

In these experiments, the 125 I-iodoglucagon (kindly provided by Dr. Bruce Frank, Eli Lilly, Indianapolis, Indiana, USA) was 95% immunoprecipitable by excess antiserum. Mononuclear cells were incubated in 0.1 mol/l HEPES-1% bovine serum albumin (pH 7.4) at 37 °C. At the indicated times triplicate 0.5 ml aliquots were taken for measurement of degradation. Cell concentration was $25 \times 10^6/0.5$ ml. Each 0.5 ml aliquot was centrifuged to precipitate the cells and from each tube 200 μ l was taken for precipitation with trichloroacetic acid and 200 μ l taken for immunoprecipitation. For determination of immunoprecipitability, 200 μ l aliquots containing 125 I iodoglucagon in 0.1 mol/l HEPES-1% bovine serum albumin were added to 200 μ l 0.15 mol/l glycine buffer (pH 8.8) containing N-ethylmaleimide (final concentration 2 mmol/l). Rabbit anti-glucagon antiserum (5 μ l) was added and the tubes incubated at 4 °C for 48 h. Normal rabbit serum (50 μ l of 1:10 dilution) and goat anti-rabbit gamma globulin (50 μ l) were added and the tubes incubated for an additional 24 h at 4 °C. The tubes were centrifuged and the amount of immunoprecipitable 125 I iodoglucagon determined. Appropriate controls containing no antiserum as well as controls containing undegraded glucagon were included.

Mononuclear Cell Homogenates and Subcellular Fractionation

Mononuclear cell preparations were homogenized in 0.35 mol/l sucrose in a glass on glass Dounce homogenizer (Arthur H. Thomas Company, Philadelphia, Pennsylvania, USA). The homogenates were then placed in a 500 μ l volume of HEPES-bovine serum albumin at pH 7.4 and incubated for 30 min at 37 °C. The reaction was terminated by addition of sufficient trichloroacetic acid to achieve a final concentration of 5%.

In other studies, subcellular fractions were obtained by centrifuging the homogenates at 500 g for 30 min. The resulting supernatant was then centrifuged at 30,000 g for 30 min and separated from the pellet. Finally, centrifugation at 100,000 g for 30 min yielded a 100,000 g pellet and cytosolic supernatant. Neutral glucagon-degrading activity was determined on each fraction using HEPES-bovine serum albumin buffer (pH 7.4). Acidic or lysosomal activity was measured after treating each fraction with NaAc (0.1 mol/l), NaCl (0.1 mol/l), 0.1% (v/v) Triton X-100 at pH 4.5 for 12 h to lyse the lysosomes [10].

K_m Determinations

Kinetic analyses were performed on glucagon-degrading activity from intact homogenized cells, and cytosol using standard 125 I-iodoglucagon (1×10^{-10} mol/l) and varying concentrations of unlabelled glucagon to obtain initial rates. The data were analysed by linear regression.

Results

Glucagon Degradation by Intact Cells

The effect of total number of mononuclear cells on the cell association and degradation of glucagon is illustrated in Figure 1. Both total cell associated radioactivity and degradation were linearly related to cell number. No degrading activity was present in the supernatant after removal of the cells.

Mononuclear cells incubated for various times between 5 and 120 min demonstrated a time-dependent increase in glucagon degradation (Fig. 2). Extensive metabolism of glucagon is reflected by the 50% degradation seen at 90 min. In these experiments, prolonged

Fig. 2. Glucagon degradation at increasing incubation times. Mononuclear cells (25×10^6 in 500 μ l buffer as described in Figure 1) were incubated for varying times at 37°C. The incubation mixture after removal of the cells degraded 20% of the total glucagon degraded by the cells

Fig. 3. Temperature effect on glucagon degradation. A volume of 500 μ l of buffer as described in Figure 1 containing 25×10^6 cells and 125 I-iodoglucagon was incubated for 30 min at the indicated temperatures. No degrading activity was present in the medium after removal of the cells

incubation resulted in leakage of degrading activity in the supernatant accounting for about 20% of the total glucagon degraded.

Glucagon degradation was also temperature dependent with very little degradation at 4°C and a sixfold increase over a range of 4°–37°C. (Fig. 3).

The effect of trichloroacetic acid precipitation on glucagon degradation is shown in Table 1. Assay of glucagon degradation by either trichloroacetic acid precipitability or by immunoprecipitability gave comparable results. These data suggest that the intact monocyte produced minimal amounts of degraded material which precipitated with trichloroacetic acid but did not react with glucagon antiserum.

Glucagon Degradation by Mononuclear Cell Homogenates

Homogenized mononuclear cell preparations degraded 10–20 times more glucagon than intact cells (see below). Figure 4 shows the effect of cell number on glucagon degradation by homogenates. Very rapid proteolysis was seen with almost complete degradation at 30 min

Table 1. A comparison of immunoprecipitation and trichloroacetic acid precipitation for assessing 125 I-iodoglucagon degradation by human monocytes

Time (min)	125 I-iodoglucagon degradation	
	Soluble in trichloroacetic acid (%)	Non-immunoprecipitable (%)
15	11.6	14.8
30	19.6	20.9
60	34.4	32.6
120	51.6	51.2

Mononuclear cells were incubated at 37°C for the indicated times. Aliquots were centrifuged to precipitate the cells and degradation measured by precipitation with trichloroacetic acid and immunoprecipitation as described in Methods

Table 2. Subcellular distribution of glucagon degrading activity in human mononuclear cell homogenates

Fraction	Glucagon degraded (fmol/l) ^a	Total activity (%)
Neutral pH		
Homogenate	499.00	100
500 g pellet	57.2	11.2
30,000 g pellet	61.6	12.1
100,000 g pellet	17.8	3.5
100,000 g supernatant	374.0	73.2
Acidic pH		
Homogenate	97.5	100
500 g pellet	151.0	155.0
30,000 g pellet	10.3	19.8
100,000 g pellet	0	0
100,000 g supernatant	0	0

^a For 5×10^6 cells at 37°C for 30 min. Glucagon concentration 10^{-9} mol/l

with high cell number concentrations. Degradation appeared to be linearly associated only at low cell numbers and the time course of glucagon degradation reached a plateau at 45 min (data not shown). Similar to the intact cells, degrading activity increased sixfold from 4°C to 37°C in the homogenates (Fig. 5).

Subcellular Distribution of Glucagon-Degrading Activity

Neutral and acidic glucagon-degrading activity was measured in the mononuclear cell homogenates as well as the 500 g, 30,000 g, and 100,000 g pellets and in the 100,000 g supernatant (cytosol). Neutral degrading activity was found in each subcellular fraction (Table 2) with 73% located in the cytosolic fraction. Total neutral activity recovered from the subcellular fractions agreed well with the quantity present in the homogenate. Acidic activity is primarily lysosomal in nature and, accordingly, the 500 g pellet contained almost all of the acidic activity with none present in the cytosolic fraction. The fact that recovery of acidic activity in the subcellular fractions was almost double that found in the homogenate is probably due to the removal of cytosolic inhibi-

Fig. 4. Glucagon degradation by varying concentrations of homogenized mononuclear cells. The homogenates were incubated with glucagon in a total volume of 500 μ l for 30 min at 37°C

Fig. 5. Effect of temperature on glucagon degradation by homogenized mononuclear cells. A volume of 500 μ l containing 5×10^6 cells was incubated for 30 min at various temperatures

Table 3. Summary of kinetic constants for glucagon degradation by intact human mononuclear cells and by homogenates and cytosolic fraction of mononuclear cells

Source of Activity	K_m ($\times 10^{-5}$ mol/l)	V_{max} (5×10^6 /30 min)	r
Intact cells	1.11	3.45×10^{-10}	0.9999
Homogenates	0.75	7.82×10^{-10}	0.9956
Cytosol	1.18	12.0×10^{-10}	0.9998

tors of lysosomal enzymes in the fractionation process. Note that the neutral glucagon-degrading activity comprised 75% of the total cellular glucagon-degrading activity in these assays.

Kinetics of Glucagon Degradation

Intact mononuclear cells, homogenized mononuclear cells, and cytosol fractions gave linear Lineweaver-Burke plots for glucagon degradation. Table 3 summarizes the kinetic constants derived by linear regression analysis. K_m values of 1.1×10^{-5} mol/l, 7.5×10^{-6} mol/l and 1.2×10^{-5} mol/l were obtained for intact cells, homogenates and cytosol, respectively. In-

Table 4. Inhibitors of glucagon degradation by intact human mononuclear cells and by cytosol from mononuclear cells

Inhibiting compound	Concentration (mmol/l)	Glucagon degrading activity (intact cells) ^a	Cytosol ^b
N-ethylmaleimide	0.2	0	0
P-hydroxy-mercuribenzoate	0.1	0	0
EDTA	1.0	46	71
	5.0	39	67
Vinblastine	0.1	73	63
	0.5	69	54
Chloroquine	0.1	86	67
	2.0	57	43
Lidocaine	1.0	94	48
	5.0	102	29
Aprotinin (Trasyolol)	500 KIU/ml	59	88
	5000 KIU/ml	66	87
Pepstatin	0.1	60	67
	0.5	56	45
Bacitracin	0.1	27	34
	1.0	25	29
Methylamine	1.0	63	69
	10.0	13	20
Dansylcadaverine	0.1	13	49
	0.5	0	22

^a Pre-incubation for 15 min, 5×10^6 at 37°C 30 min. All solutions (pH 7.4) after addition of all components; ^b 1×10^6 cell equivalents, same conditions

sulin was found to be a competitive inhibitor of the degradation of glucagon by intact mononuclear cells with a K_i value of 3.6×10^{-7} mol/l (Fig. 6).

Inhibitor Studies

To ascertain the nature of the cellular degrading enzymes, intact cells and cytosol fractions were examined for sensitivity to various inhibitors (Table 4). N-ethylmaleimide and p-hydroxymercuribenzoate totally inhibited glucagon degradation by both intact cells and cytosol, indicating that the enzymatic systems of both are sulphydryl-dependent. An apparent effect was also seen with EDTA. Chloroquine and lidocaine, known lysosomotropic agents, inhibited degradation by the cytosol, while only chloroquine was effective against intact cells. These data are difficult to explain as lysosomotropic effects since the cytosol had no detectable lysosomal activity. Vinblastine, a compound which inhibits microtubule formation, inhibited degradation as did aprotinin and pepstatin (general protease inhibitors). Bacitracin, methylamine and dansylcadaverine functioned analogously to inhibit glucagon degradation by intact cells and cytosolic fraction alike. With the exception of lidocaine, all 12 inhibitors had the same effect on the cytosol as on the intact cells, indicat-

Fig. 6. Double reciprocal plot of glucagon degradation by intact mononuclear cells with and without 2×10^{-7} mol/l insulin. Varying concentrations of unlabelled glucagon and tracer concentrations (1×10^{-10} mol/l) of 125 I-iodoglucagon were incubated with 10×10^6 mononuclear cells in the presence or absence of unlabelled insulin (2×10^{-7} mol/l) at 37°C for 30 min. ○ = with insulin; ● = without insulin

Table 5. Effect of inhibitors on cell-associated radioactivity in human mononuclear cells

Compound	Concentration (mmol/l)	Cell-associated radioactivity	Change in binding (%)
None	—	2.83	—
p-hydroxy-mercuribenzoate	0.1	2.73	— 3.5
N-ethylmaleimide	0.2	3.36	+ 19
Bacitracin	0.1 mg/ml 1.0 mg/ml	1.80 1.40	— 36 — 51
Methylamine	1.0 10	3.50 3.90	+ 24 + 38
Dansylcadaverine	0.1 0.5	2.61 2.52	— 8 — 11
Chloroquine	0.1 2.0	4.11 4.09	+ 45 + 45
Lidocaine	5	3.30	+ 17

^a Pre-incubation for 15 min, 25×10^6 cells, 30 min 37°C

Table 6. Glucagon degradation by intact and homogenized human mononuclear cells

Preparation	Glucagon binding ^a (%)	Glucagon degraded (mol/l) ^a
Intact mononuclear cells	2	3.73×10^{-14}
Intact monocytes	>2	16.10×10^{-14}
Intact lymphocytes	<1	1.90×10^{-14}
Homogenized mononuclear cells	—	38.90×10^{-14}
Homogenized monocytes (97% pure)	—	53.30×10^{-14}
Homogenized lymphocytes (98% pure)	—	9.00×10^{-14}

^a Per 5×10^6 cells at 37°C for 30 min. Glucagon concentration 10^{-9} mol/l

ing the importance of cytosolic enzymes in the metabolism of glucagon by intact mononuclear cells.

Several investigators have noted an accumulation of cell-associated radioactivity on target cells treated with enzymatic inhibitors presumably due to the accumulation of undegraded hormone [11, 12]. Chloroquine and lidocaine gave a 45% and 17% increase in cell-associated radioactivity, respectively (Table 5). Similar results were obtained by Hofmann et al., using insulin and cultured hepatoma cells [13]. Bacitracin, methylamine, and dansylcadaverine did not increase consistently cellular radioactivity. In fact, no correlation was seen between the potency of the inhibitor and the accumulation of cellular radioactivity as shown in Table 5.

Glucagon Degradation by Monocytes and Lymphocytes

To examine the relative roles of monocytes and lymphocytes in glucagon degradation, highly purified preparations were obtained. A 98% pure preparation of intact lymphocytes did not degrade significant quantities of glucagon. Monocytes (97% pure) were very active in metabolizing glucagon with 3.73×10^{-14} mol/l degraded in 30 min by 5×10^6 cells.

Homogenized mononuclear cells exhibited 10–20 times the degrading activity of intact cells, indicating that the bulk of the degrading activity was intracellular. However, homogenized monocyte preparations (enriched four to five times) were only two to three times more active in degrading glucagon than intact monocytes (Table 6). A 98% pure lymphocyte preparation exhibited significant glucagon-degrading activity only when homogenized.

Discussion

Monocytes have been reported to be responsible for specific binding of glucagon in mononuclear cell preparations [5]. In the present study, we have demonstrated that mononuclear cells in general and monocytes in particular degrade glucagon in a time, temperature, and cell-concentration-dependent manner. Subcellular fractionation of mononuclear cells revealed that 73% of the neutral glucagon-degrading activity was in the cytosol, as previously reported for insulin-degrading activity [15]. Quantitatively, neutral activity was three times greater than acidic activity as reported also for liver [16]. Glucagon degrading activity of both intact cells and cytosol fractions was susceptible to the same inhibitors indicating that glucagon degradation by intact cells was a function of cytosolic enzymes, again similar to the liver [16], but different from kidney [17, 18]. A cytosolic enzyme has been isolated from various animal tissues which degrades both insulin and glucagon [19, 20]. This soluble neutral protease has properties very similar to those of the glucagon degrading activity found in hu-

man mononuclear cells and may well be the same enzyme.

Kinetic studies gave similar K_m values for glucagon degradation by intact mononuclear cells, homogenates and cytosol. These values are in the same range as those previously reported for liver and kidney membranes [21] and also similar to the values obtained for insulin protease from rat tissues [18, 19]. Recently, a proteolytic cytosolic enzyme has been isolated from human skeletal muscle which degrades glucagon ($K_m = 1.3 \times 10^{-5}$ mol/l) and is competitively inhibited by insulin ($K_i = 5 \times 10^{-8}$ mol/l) [22]. Significantly, glucagon degradation by intact mononuclear cells was competitively inhibited by insulin with a K_i value of 3×10^{-7} mol/l. These data suggest that the cytosolic glucagon-degrading system may be identical to that functioning in intact cells and that this enzyme is similar to insulin protease.

Several investigators have suggested that lysosomes are involved in insulin degradation, both from morphological studies and from studies of lysosomotropic agents such as chloroquine [12, 13, 23]. As reported with insulin, we also found increased cell-associated radioactivity and decreased glucagon degradation with lysosomotropic compounds. However, cytosolic glucagon-degrading activity, which is devoid of lysosomal activity, was similarly inhibited by lysosomotropic agents such as chloroquine. These data may imply that the cellular degradation of glucagon in the monocyte, although susceptible to lysosomotropic agents, is primarily mediated by neutral cytosolic proteases or at least by proteases which fractionate into the 100,000 g supernatant. Goldstein and Livingston have reached the same conclusion for insulin degradation by rat adipocytes [24].

Davies et al. have recently drawn a correlation between the inhibition of transglutaminase and the inhibition of receptor mediated endocytosis [25, 26]. The transglutaminase inhibitors, bacitracin, methylamine, and dansylcadaverine, were tested for their effects on cell-associated radioactivity and on glucagon degradation by mononuclear cells. All three compounds effectively inhibited degradation of glucagon by both intact cells and cytosol but only methylamine gave an increase in cell-associated radioactivity. These data suggest that transglutaminase inhibitors may have multiple cellular effects as reported previously [27].

In summary, glucagon is rapidly degraded by human monocytes. Cellular glucagon degradation appears to be primarily mediated by a neutral cytosolic sulphhydryl-dependent protease(s). This enzyme(s) is similar in the following properties to insulin protease previously identified in other tissue: (1) subcellular location, (2) K_m for glucagon degradation, (3) K_i for insulin, and (4) sensitivity to a wide range of inhibitors.

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References

1. Exton JM, Park CR (1968) The role of cyclic AMP in the control of liver metabolism. *Adv Enzyme Regul* 6: 391-407
2. Rodbell M (1972) Regulation of glucagon action on its receptors, In: Lefvre PJ, Unger PJ (eds) *Glucagon: molecular physiology, clinical and therapeutic implications*, Pergamon Press, Oxford, pp 61-75
3. Sutherland EW, Robinson GA, Butcher RW (1968) Some aspects of the biological role of adenosine 3', 5'-monophosphate (cyclic AMP). *Circulation* 37: 279-306
4. Goldstein SM, Blecher M, Binder R, Perrino PV, Recant L (1975) Hormone receptors: binding of glucagon and insulin to human circulating mononuclear cells in diabetes mellitus. *Endocr Res Commun* 2: 367-376
5. Bhatthana S, Louie J, Schechter GP, Redman RS, Wahl L, Recant L (1981) Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. *Diabetes* 30: 127-131
6. Boyum A (1968) Separation of leukocytes from blood and bone marrow. IV. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 21 (Suppl 97): 77-89
7. Yam LT, Li CT, Crosby WH (1971) Cytochemical identification of monocytes and granulocytes. *Am J Clin Pathol* 55: 283-290
8. Ly I, Mishell RI (1974) Separation of mouse spleen cells by passage through columns of sephadex G-10. *J Immunol Methods* 5: 239-244
9. Huber TH, Solomon SS, Duckworth WC (1980) The time course of insulin degradation in the perfused isolated fat cell. *J Clin Invest* 65: 461-468
10. Weissman B, Rowin G, Marshall T, Frederici D (1967) Mammalian alpha-acetylglucosaminidase. Enzyme properties, tissue distribution and intracellular localization. *Biochem* 6: 207-214
11. Marshall S, Olefsky JM (1979) Effects of lysosomotropic agents on insulin interaction with adipocytes. *J Biol Chem* 253: 10153-10162
12. Hammons GT, Jarett L (1980) Lysosomal degradation of receptor bound 125 I-labeled insulin by rat adipocytes. *Diabetes* 29: 475-486
13. Hofmann C, Marsh JW, Miller B, Steiner DF (1980) Cultured hepatoma cells as a model system for studying insulin processing and biological responsiveness. *Diabetes* 29: 865-874
14. Gambhir KK, Nerurkar SG, Das PD, Arker JA, Henry WL Jr. (1981) Insulin binding and degradation by human erythrocytes at physiological temperature. *Endocrinology* 109: 1787-1789
15. Powers AC, Solomon SS, Duckworth WC, (1980) Insulin degradation by mononuclear cells. *Diabetes* 29: 27-32
16. Ansorge S, Bohley P, Kirschke H, Langner J, Hanson H (1971) Metabolism of insulin and glucagon. *Eur J Biochem* 19: 283-288
17. Duckworth WC (1976) Insulin and glucagon degradation by the kidney. I. Subcellular distribution under different assay conditions. *Biochim Biophys Acta* 437: 518-530
18. Duckworth WC (1976) Insulin and glucagon degradation by the kidney. II. Characterization of the mechanisms at neutral pH. *Biochim Biophys Acta* 437: 531-542
19. Duckworth WC, Kitabchi AE (1974) Insulin and glucagon degradation by the same enzyme. *Diabetes* 23: 536-543
20. Duckworth WC, Heinemann M, Kitabchi AE (1975) Proteolytic degradation of insulin and glucagon. *Biochim Biophys Acta* 377: 421-430
21. Duckworth WC (1975) Insulin and glucagon binding and degradation by kidney cell membranes. *Endocrinology* 102: 1766-1772
22. Neal GW, Kitabchi AE (1982) Insulin degradation by human skeletal muscle. *Biochim Biophys Acta* 719: 259-266

23. Barrazone P, Gorden P, Carpentier JL, Orci L, Freychet P, Canivet B (1980) Binding, internalization and lysosomal association of 125 I-glucagon in isolated rat hepatocytes: a quantitative electron microscope autoradiographic study. *J Clin Invest* 66: 1081–1093
24. Goldstein HJ, Livingston JN (1981) An evaluation of the importance of lysosomal and neutral cytosol proteases in insulin degradation by adipocytes. *Endocrinology* 108: 953–961
25. Davies PJA, Davies DR, Levitski A, Maxfield FR, Milhaud P, Willingham MC, Pastan IH (1980) Transglutaminase is essential in receptor-mediated endocytosis of 2-macroglobulin and polypeptide hormones. *Nature* 283: 162–167
26. Maxfield FR, Davies PJA, Klempner L, Willingham MC, Pastan IH (1979) Epidermal growth factor stimulation of DNA synthesis is potentiated by compounds that inhibit its clustering in coated pits. *Proc Natl Acad Sci* 76: 5731–5735
27. Ahmed CE, Niswender GD (1981) Internalization and degradation of human chorionic gonadotropin in ovine luteal cells: Effects of inhibitors of transglutaminase. *Endocrinology* 109: 1388–1393

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