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# Differential Centrifugation Studies of Guinea Pig Lung Proteases (40323)

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Recent literature has indicated the presence of serveral cathepsins in lung tissue. Using crude preparations, Otto (1) reported that several rat organs, including lung, contained cathepsins B1 and B2. McDonald and co-workers (2), using aqueous extracts of a number of rat tissues and employing highly specific synthetic substrates, reported that lung contained dipeptidylpeptidase I (cathepsin C), II, III, and IV. Finally, cathepsin D has been isolated and purified from extracts of homogenized rabbit and beef lung (3). Relatively little is known about the properties of these proteolytic enzymes in this tissue, or about their distribution among the subcellular fractions of lung. A preliminary study of rabbit lung (4) indicated that the subcellular fractions prepared were heterogeneous, that some enzyme markers were widely distributed among the fractions, and that classical lysosomal enzymes appeared to be distributed differently in lung than in liver. With lung possessing several different kinds of cells, one might expect a heterogeneity of organellar size, and consequently a wider distribution of marker enzymes in particles of varying size.

Our interest in proteolytic enzymes in lung, under normal (5, 6) and pathological (7) conditions, has prompted us to examine the distribution of 18 enzymes among five subcellular fractions prepared by differential pelleting. Seven marker enzymes and eleven proteolytic enzymes were examined to lay a basis for a more detailed examination of lung lysosomes and lysosomal proteases and peptidases.

*Materials and methods. Disruption and subcellular fractionation.* Lungs were obtained from 500-g short-haired, outbred guinea pigs from a local colony. The lungs were perfused (via the pulmonary artery) with 200 to 300 ml of saline to remove all blood, minced, and then suspended in cold 0.25 *M* sucrose solution (pH 7.2). The suspended material was disrupted by brief homogenization, achieved by five up-and-down strokes of a motor-driven (1000 rpm) Potter–Elvehjem homogenizer (clearance, 4–6 μm). This homogenate protocol consistently resulted in high yields of intact lysosomes. The homogenate was brought to 10% (w/v) with 0.25 *M* sucrose and was filtered through cheesecloth prior to centrifugation. Fractionation of the homogenates was achieved by 5-fraction differential centrifugation following the procedure for liver (8) without modification. These fractions were: nuclear, N (510*g* × 10 min); heavy mitochondrial, M (10,000*g* × 5 min); light mitochondrial, L (40,000*g* × 10 min); microsomal, P (100,000*g* × 45 min); and soluble, S (nonsedimentable). Total activity of 18 enzymes was investigated in each of these fractions after treatment with Triton X-100 (Sigma). The overall concentration of Triton X-100 was 0.2% (w/v). It was used to release membrane-bound enzyme activities. This low level of Triton did not affect any of the enzyme assays.

*Enzyme analysis.* All concentrations given are final concentrations in the assay mixture. Cytochrome oxidase and cathepsin D were assayed as described by Canonico and Bird (9). Lactate dehydrogenase was detected using Sigma Kit 500. *N*-Acetyl-β-glucosaminidase was detected using the (0.02 *M*) *p*-nitrophenyl derivative (Sigma) in 0.1 *M* acetate buffer, pH 5.0. The reaction was stopped with 1.25 *N* NaOH and filtered (Whatman No. 42), and the absorbance was read at 440 nm on a Gilford spectrophotometer. Acid *p*-nitrophenylphosphatase was assayed as described by Bosmann and Hemsworth (10). Alkaline *p*-nitrophenylphosphatase was determined by the procedure of Garen and Levinthal (11) except that the pH was held at 8.8 (where the color is somewhat more intense) rather than at 8.0. Succinate dehydro-

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genase was measured using the method of Pennington (12) in which the dye 2-(*p*-iodophenyl-3-*p*-nitrophenyl)-5-phenyl-tetrazolium was reduced by succinate to produce formazan which was extracted into ethyl acetate and read at 490 nm. Glucose 6-phosphatase was determined by the method of Nordlie and Arion (13) using the sodium cacodylate buffer, pH 6.5. Inorganic phosphorus was determined by the method of Chen *et al.* (14). Cathepsin A was measured using the method of Iodice *et al.* (15) with *N*-carbobenzoxy-$\alpha$-glutamyl-L-tyrosine (Cyclo Chemical Co., Los Angeles, California)[2] as substrate. The rate of production of free amino groups was monitored with the ninhydrin reagent of Moore and Stein (16). To analyze the cathepsin B1, the method of Barrett (17) was employed. Dipeptidylpeptidases I, II, III, and IV were determined using the method of McDonald *et al.* (18). The substrates used for the fluorimetric assays were as follows: dipeptidylpeptidase I, 0.1 m*M* Gly–Arg–$\beta$-naphthylamide (2) in 5 m*M* NaCl–7.5 m*M* 2-mercaptoethanol–70 m*M* sodium succinate, pH 5.0; dipeptidylpeptidase II, 0.2 m*M* Lys–Ala–$\beta$-naphthylamide in 10 m*M* 3,3-dimethylglutaric acid, pH 5.5; dipeptidylpeptidase III, 0.03 m*M* Arg–Arg–$\beta$-naphthylamide in 62.5 m*M* Tris–HCl, pH 9.0; dipeptidylpeptidase IV, 0.17 m*M* Gly–Pro–$\beta$-naphthylamide in 20 m*M* Tris–HCl, pH 7.8. Calibration was carried out with known standards of $\beta$-naphthylamine. All $\beta$-naphthylamides were purchased from Bachem (Torrance, California). For the analysis of elastolytic esterase, the method of Visser and Blout (19) was used. In this procedure, 0.33 m*M* *p*-nitrophenyl *N*-tert-butyloxycarbonyl-L-alanate (Sigma) was used as substrate in 0.05 *M* sodium phosphate–3% acetonitril, pH 6.5. Dipeptidase was assayed using the titrimetric assay of Bryce and Rabin (20). Glycyl-L-leucine was used as the substrate. A radiometer titrigraph Type SBR 2c was used to keep the pH constant at 8.4 by adding standardized acid. Neutral and alkaline protease activities were measured on 1% heat-denatured casein solutions at pH 7.0 and 8.5, respectively, similar to the method of Kunitz (21). After 30 min, 10% trichloroacetic acid was used to precipitate proteins and large peptide fragments. The absorbance of the supernatant at 280 nm was used as an indication of protease activity.

Protein was determined by the Biuret method of Gornall *et al.* (22) using bovine serum albumin Fraction V (Sigma) as standard.

*Presentation of results.* To simplify construction of tables and graphs, the following symbols were used: N = nuclear fraction; M = heavy mitochondrial fraction; L = light mitochondrial or lysosomal fraction; P = microsomal fraction; S = final supernatant or cytoplasmic fraction.

The percentage of an enzyme in any one fraction was determined by dividing the activity in that fraction by the total activity obtained in the five fractions × 100. The percentage recovery was determined by dividing the sum of an enzyme's activity in the five fractions, N, M, L, P, and S, by the activity determined on a sample of homogenate prior to centrifugation, × 100.

The relative specific activity in each fraction was obtained as follows: percentage of total activity/percentage of total protein × 10, according to de Duve *et al.* (8).

The distributions of the enzymes' activities after differential centrifugation are presented by plotting the mean relative specific activity against the protein content of each fraction. The area of each block represents the percentage of the total activity recovered in that fraction, and the height corresponds to the degree of purification achieved (8).

Enzyme specific activities are presented in milliunits per milligram of protein where 1 unit equals 1 $\mu$mole of substrate hydrolyzed, or 1 unit of absorbance released at 280 nm, per minute at 37°C. The units for cytochrome oxidase are calculated according to Cooperstein and Lazarow (23).

*Results. Enzyme distribution following differential centrifugation.* The distribution of 18 enzymes and of the lung protein following differential centrifugation are presented in Table I, along with the percentage of each enzyme recovered. Despite the heterogeneity of the lung cell populations, the distribution recorded for the various enzymes paralleled that found in liver. For example, the major

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[2] Cyclo Chemical Company's inventory has been purchased by Vega-Fox Biochemicals, Tuscon, Arizona.

TABLE I. PERCENTAGE OF TOTAL ACTIVITY IN TISSUE FRACTIONS.[a]

| Enzymes | Fraction | | | | | Percentage enzyme recovered |
|---|---|---|---|---|---|---|
| | N | M | L | P | S | |
| Cytochrome oxidase (1) | 17.3 | 65.6 | 16.4 | 0.7 | 0 | 85.5 |
| Succinate dehydrogenase (3) | 12.1 ± 6.8 | 39.2 ± 22.4 | 25.5 ± 15.6 | 12.7 ± 12.1 | 10.5 ± 12.6 | 121.7 ± 48.3 |
| Lactate dehydrogenase (3) | 8.6 ± 6.2 | 3.7 ± 3.4 | 3.1 ± 0.6 | 7.7 ± 1.9 | 76.9 ± 8.1 | 79.2 ± 8.6 |
| *N*-Acetyl-β-glucosaminidase (1) | 32.0 | 32.4 | 15.4 | 4.6 | 15.4 | 93.4 |
| Acid *p*-nitrophenylphosphatase (3) | 22.6 ± 10.9 | 14.5 ± 5.7 | 21.5 ± 5.5 | 19.8 ± 5.3 | 21.5 ± 1.8 | 96.0 ± 6.4 |
| Alkaline *p*-nitrophenylphosphatase (3) | 4.8 ± 5.1 | 13.6 ± 5.7 | 20.9 ± 4.5 | 29.3 ± 5.6 | 31.1 ± 9.2 | 69.9 ± 31.9 |
| Glucose 6-phosphatase (3) | 13.2 ± 6.0 | 9.8 ± 4.1 | 18.3 ± 4.8 | 25.8 ± 1.7 | 32.8 ± 8.6 | 198.6 ± 131.0 |
| Cathepsin A (3) | 19.0 ± 13.6 | 5.2 ± 5.2 | 31.6 ± 14.1 | 4.6 ± 6.3 | 39.7 ± 3.7 | 62.3 ± 24.2 |
| Cathepsin B1 (3) | 24.5 ± 13.9 | 15.2 ± 21.0 | 39.5 ± 24.4 | 3.5 ± 4.8 | 17.2 ± 8.4 | 362.5 ± 417.7 |
| Cathepsin D (3) | 17.5 ± 4.8 | 20.5 ± 5.8 | 20.8 ± 5.7 | 7.6 ± 2.8 | 33.6 ± 4.4 | 142.2 ± 29.9 |
| Dipeptidylpeptidase I (3) | 13.9 ± 17.1 | 8.9 ± 1.6 | 25.6 ± 4.8 | 3.4 ± 2.9 | 48.2 ± 12.8 | 68.4 ± 12.8 |
| Dipeptidylpeptidase II (3) | 18.7 ± 8.8 | 24.1 ± 9.0 | 20.2 ± 5.6 | 4.2 ± 3.2 | 32.7 ± 9.9 | 114.5 ± 33.6 |
| Dipeptidylpeptidase III (3) | 1.8 ± 1.1 | 5.9 ± 9.0 | 4.4 ± 4.7 | 2.6 ± 0.7 | 85.3 ± 15.2 | 147.5 ± 12.2 |
| Dipeptidylpeptidase IV (3) | 16.6 ± 14.9 | 9.4 ± 3.8 | 22.1 ± 4.9 | 33.5 ± 13.8 | 18.4 ± 3.3 | 113.2 ± 16.2 |
| Elastolytic esterase (3) | 11.9 ± 7.3 | 9.2 ± 3.7 | 15.5 ± 3.4 | 10.5 ± 2.7 | 52.9 ± 6.5 | 99.2 ± 15.8 |
| Neutral protease (3) | 3.9 ± 3.8 | 5.5 ± 3.8 | 11.3 ± 2.0 | 29.7 ± 13.1 | 49.5 ± 21.5 | 139.2 ± 30.2 |
| Alkaline protease (3) | 2.8 ± 3.9 | 5.3 ± 4.6 | 11.4 ± 1.0 | 31.1 ± 13.2 | 49.4 ± 20.3 | 151.2 ± 16.3 |
| Dipeptidase (1) | 1.3 | 0.7 | 1.3 | 1.8 | 95.0 | 118.1 |
| Protein (3) | 21.3 ± 12.2 | 13.2 ± 3.3 | 9.9 ± 4.0 | 11.5 ± 4.5 | 44.2 ± 7.2 | 104.5 ± 4.7 |

[a] Values indicate the mean percentage ± the standard deviation of the mean. Numbers in parentheses indicate the number of experiments. The percentage enzyme activity and percentage enzyme recovered were calculated as described in the section under Experimental.

part of the activities of both cytochrome oxidase and succinate dehydrogenase is found in the heavy mitochondrial fraction, and of lactate dehydrogenase in the supernatant fraction, as expected.

The percentage recovery of nine enzymes (cytochrome oxidase, succinate dehydrogenase, lactate dehydrogenase, *N*-acetyl-β-glucosaminidase, acid-*p*-nitrophenylphosphatase, dipeptidylpeptidase II and IV, elastolytic esterase, and dipeptidase) and of protein were good (i.e., 79–122%); the recoveries of three enzymes (alkaline *p*-nitrophenylphosphatase, cathepsin A, and dipeptidylpeptidase I) were low (62–70%) whereas six enzymes (glucose 6-phosphatase, cathepsins B1 and D, dipeptidylpeptidase III, and neutral and alkaline protease) showed significantly greater total activity in the sum of the fractions than in the whole homogenate (Table I). It is possible that fractionation removed some inhibitor of these enzymes and allowed for greater expression of total activity in the fractions. This has already been demonstrated in our laboratory, where the addition of a small aliquot of the supernatant fraction to the light mitochondrial fraction strongly inhibited cathepsin B1 activity, as measured by the hydrolysis of benzoyl-arginyl-β-naphthylamide (24).

*Relative specific activities.* The relative specific activity of each enzyme in each of the five tissue fractions is presented in Table II. These values were plotted vs the percentage protein in each fraction to give the graphs which are presented in Fig. 1.

Cytochrome oxidase and succinate dehydrogenase, two mitochondrial markers, were enriched in the heavy mitochondrial fraction, M, and to a lesser extent in the light mitochondrial fraction, L.

*N*-Acetyl-β-glucosaminidase, acid *p*-nitrophenylphosphatase, dipeptidylpeptidase I, dipeptidylpeptidase II, cathepsin A, cathepsin B1, cathepsin D, and elastolytic esterase all showed greatest enrichment in the light mitochondrial fraction, L. Among these enzymes there appeared to be two separate patterns of distribution. Cathepsin A, cathepsin B1, and dipeptidylpeptidase I appeared to distribute so that the light mitochondrial fraction was greatly enriched over the neighboring fractions. On the other hand, *N*-acetyl-β-glucosaminidase, cathepsin D, dipeptidylpeptidase II, acid *p*-nitrophenylphosphatase, and elastolytic esterase distributed throughout the fractions such that the light mitochondrial fraction, L, was only slightly enriched over the neighboring fractions. In this second class of enzymes, the distribution throughout the fractions seemed to be broader than the first class.

The microsomal fraction, P, was enriched in glucose 6-phosphatase, alkaline *p*-nitro-

TABLE II. RELATIVE SPECIFIC ACTIVITIES IN TISSUE FRACTIONS.[a]

| Enzyme | Fraction | | | | |
|---|---|---|---|---|---|
| | N | M | L | P | S |
| Cytochrome oxidase (1) | 0.7 | 5.2 | 3.9 | 0.1 | 0 |
| Succinate dehydrogenase (3) | 0.9 ± 0.8 | 3.8 ± 1.4 | 3.5 ± 1.1 | 1.5 ± 1.2 | 0.3 ± 0.4 |
| Lactate dehydrogenase (3) | 1.1 ± 0.3 | 0.6 ± 0.2 | 1.0 ± 0.3 | 2.0 ± 0.3 | 5.1 ± 0.6 |
| *N*-Acetyl-β-glucosaminidase (1) | 1.3 | 2.8 | 4.2 | 1.0 | 0.6 |
| Acid *p*-nitrophenylphosphatase (3) | 1.6 ± 0.7 | 1.7 ± 0.8 | 3.3 ± 0.4 | 2.6 ± 0.4 | 0.7 ± .03 |
| Alkaline *p*-nitrophenylphosphatase (3) | 0.5 ± 0.5 | 1.4 ± 0.2 | 3.2 ± 0.4 | 3.9 ± 0.5 | 1.0 ± 0.3 |
| Glucose 6-phosphatase (3) | 1.1 ± 0.6 | 1.3 ± 0.8 | 2.9 ± 0.2 | 3.7 ± 0.9 | 1.1 ± 0.3 |
| Cathepsin A (3) | 1.7 ± 1.6 | 0.6 ± 0.5 | 5.5 ± 0.8 | 0.6 ± 0.7 | 1.6 ± 0.5 |
| Cathepsin B1 (3) | 2.0 ± 1.7 | 1.2 ± 1.5 | 5.7 ± 1.7 | 0.4 ± 0.5 | 0.6 ± 0.3 |
| Cathepsin D (3) | 1.5 ± 0.4 | 2.6 ± 1.0 | 3.6 ± 0.5 | 1.1 ± 0.4 | 1.2 ± 0.3 |
| Dipeptidylpeptidase I (3) | 0.7 ± 0.5 | 1.4 ± 0.7 | 5.2 ± 0.8 | 0.5 ± 0.3 | 2.1 ± 0.8 |
| Dipeptidylpeptidase II (3) | 1.6 ± 0.4 | 3.1 ± 0.7 | 3.6 ± 0.5 | 0.6 ± 0.3 | 1.2 ± 0.3 |
| Dipeptidylpeptidase III (3) | 0.3 ± 0.3 | 0.8 ± 0.9 | 1.5 ± 1.6 | 0.8 ± 0.2 | 6.6 ± 2.6 |
| Dipeptidylpeptidase IV (3) | 1.0 ± 0.2 | 1.0 ± 0.1 | 3.4 ± 0.7 | 5.1 ± 2.4 | 0.6 ± 0.2 |
| Elastolytic esterase (3) | 1.1 ± 0.4 | 1.4 ± 0.8 | 3.3 ± 0.8 | 1.9 ± 0.5 | 2.3 ± 0.5 |
| Neutral protease (3) | 0.5 ± 0.5 | 0.8 ± 0.7 | 2.1 ± 0.5 | 4.5 ± 0.7 | 2.0 ± 1.1 |
| Alkaline protease (3) | 0.4 ± 0.5 | 0.9 ± 0.8 | 2.2 ± 0.6 | 4.6 ± 0.7 | 2.0 ± 0.9 |
| Dipeptidase (1) | 0.5 | 0.3 | 0.6 | 0.5 | 8.1 |

[a] Values are the mean relative specific activity ± standard deviation of the mean. Numbers in parentheses are the number of experiments.

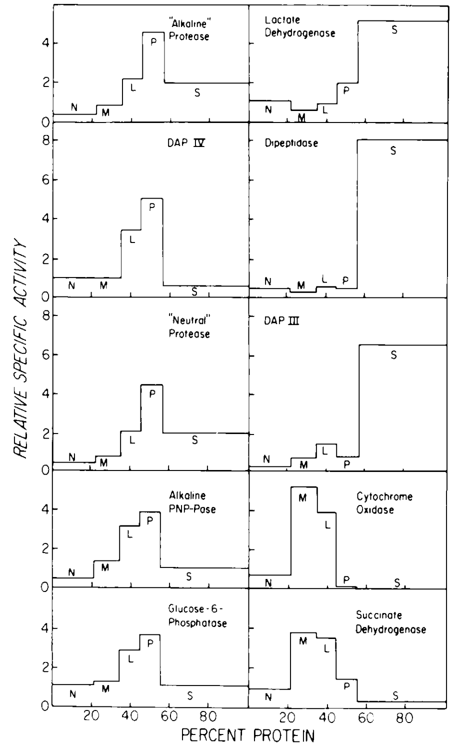

FIG. 1. Distribution patterns of enzymes after differential centrifugation. Fractions are N, nuclear; M, heavy mitochondrial; L, light mitochondrial; P, microsomal; and S, nonsedimentable. Enzyme abbreviations are DAP, dipeptidylaminopeptidase or dipeptidylpeptidase; PNP-Pase, *p*-nitrophenylphosphatase.

phenylphosphatase, neutral protease, dipeptidylpeptidase IV, and alkaline protease. For these enzymes considerable activity was also found in the light mitochondrial fraction.

Three enzymatic activities were found in the cytosol: lactate dehydrogenase, dipeptidase, and dipeptidylpeptidase III. The specific activities for the 18 enzymes in guinea pig lung are presented in Table III.

*Discussion.* This study of differential centrifugation, combined with the biochemical analysis of marker enzymes, satisfies the criteria of de Duve *et al.*, (8) for separation of organelles. The fact that the mitochondrial enzymes, cytochrome oxidase and succinate dehydrogenase, the lysosomal enzyme, *N*-acetyl-β-glucosaminidase, the microsomal enzyme, glucose 6-phosphatase, and the cytosol enzyme, lactate dehydrogenase, were enriched in the fractions M, L, P, and S, respectively, indicated that the experimental procedure employed was capable of resolving to some degree the designated subcellular organelles. The somewhat broad distributions observed with these markers indicated that the fractions were heterogeneous in the organelles they contained. This was confirmed

TABLE III. SPECIFIC ACTIVITIES OF VARIOUS LUNG ENZYMES.[a]

| | |
|---|---|
| Cytochrome oxidase (5) | 50.2 ± 7.8 |
| Succinate dehydrogenase (4) | 12.3 ± 2.2 |
| Cathepsin A (4) | 46.3 ± 20.4 |
| Cathepsin B1 (4) | 3.4 ± 0.6 |
| Dipeptidylpeptidase I (2) | 10.8 |
| Cathepsin D (5) | 11.7 ± 2.7 |
| Elastolytic esterase (4) | 31.7 ± 2.0 |
| Dipeptidylpeptidase II (2) | 1.2 |
| *N*-Acetyl-β-glucosaminidase (5) | 5.2 ± 1.7 |
| Acid *p*-nitrophenylphosphatase (5) | 5.3 ± 1.7 |
| Alkaline *p*-nitrophenylphosphatase (2) | 12.0 |
| Glucose 6-phosphatase (2) | 1.6 |
| Dipeptidylpeptidase IV (2) | 2.9 |
| Alkaline protease (2) | 4.0 |
| Neutral protease (2) | 5.1 |
| Dipeptidylpeptidase III (2) | 3.1 |
| Dipeptidase (1) | 1086.0 |
| Lactate dehydrogenase (2) | 402.0 |

[a] The values (obtained with the whole homogenate) are milliunits per milligram of protein ± the standard error of the mean. The numbers in parentheses are the number of experiments.

by microscopic examination. The broad distributions seen in guinea pig lung were also noticed in rabbit lung (4).

Two other enzymes were used as secondary markers. Acid *p*-nitrophenylphosphatase were used as a lysosomal marker in spite of the fact that isoenzymes of the true acid phosphatase exist in different parts of the cell (25). While the distribution of *p*-nitrophenylphosphatase activity was broad, it did correspond to that of a lysosomal enzyme. Alkaline *p*-nitrophenylphosphatase was also used as a secondary marker of the "microsomes" since this enzyme has generally been regarded as being a component of the plasma membrane (26). Fragmented plasma membranes of rat kidney (27) and liver (28) have been found to sediment with the microsomal fraction.

The distribution of the dipeptidylpeptidase (also called dipeptidylaminopeptidase) enzymes in lung parallels their distributions in other tissues. Dipeptidylpeptidase I (or dipeptidylaminopeptidase I or cathepsin C) has been localized in rat liver (2) and bovine spleen (2). A cytosol distribution was noted for dipeptidylpeptidase III from bovine anterior pituitary (2). Dipeptidylpeptidase IV has been shown to have a microsomal distribution in porcine kidney (2) and rat liver (2).

A number of proteolytic enzymes has been localized in subcellular fractions in tissues other than lung, largely using the technique of differential centrifugation. Cathepsin A and another carboxypeptidase-like enzyme appeared to be found in the heavy mitochondrial fraction (29); cathepsins B (29) and D (30) were lysosomal in origin; and di- and tripeptidases in different tissues have been variously reported as being in the supernatant (29) and in the microsomal fractions (31, 32). Lung cathepsins A, B1, and D all appeared to be lysosomal in nature. The lung dipeptidase activity was found to be clearly associated with the cytosol fraction, as assayed with Gly–L-Leu, Gly–Gly, Gly–DL-Phe, or Gly–DL-Ser. Of eight dipeptides tested with this enzyme, the most effective substrate was Gly–L-Leu, the data for which are reported here. No tripeptidase activity against Gly–Gly–Gly or L-Leu–Gly–Gly was noted.

Elastolytic esterase was determined by the rate of breakdown of a synthetic substrate, *p*-nitrophenyl *N*-tert-butyloxycarbonyl-L-alanate. The enzyme present could not be detected using the orcein–elastin assay (33). The failure to react with the latter substrate could have been due to extremely low levels of elastase or to the fact that this enzyme was not a true elastase. Such an enzyme has been recently characterized from human pancreas (34). We have, therefore, chosen to call the enzyme measured, elastolytic esterase. This enzyme was enriched most in the light mitochondrial fraction, L, but was also present in the cytosol in sizeable quantities. The possibilities of a dual location of the same enzyme or of two different enzymes remain for consideration.

Neutral and alkaline proteases distributed with the microsomal enzyme markers. The similarity of distribution and the method of assay of the two proteases would leave open the possibility that the same enzyme is being measured at two different pH values.

The distribution of enzymes noted in this work does not differ significantly from the distributions of similar enzymes in other tissues. Further work performed on guinea pig lung using isopycnic-zonal centrifugation to obtain better resolution of fractions will be reported.

*Summary.* Five subcellular fractions were isolated from guinea pig lung homogenates by differential centrifugation. These fractions

were defined biochemically by the analysis of 18 enzymes representing different subcellular compartments. Succinate dehydrogenase and cytochrome oxidase distributed with the heavy mitochondrial fraction, while *N*-acetyl-β-glucosaminidase, acid *p*-nitrophenylphosphatase, cathepsins A, B1, and D, dipeptidylpeptidases I and II, and elastolytic esterase distributed with the light mitochondrial fraction. Alkaline *p*-nitrophenylphosphatase, glucose 6-phosphatase, dipeptidylpeptidase IV, neutral protease, and alkaline protease all demonstrated a "microsomal" enrichment. In the cytosol were found lactate dehydrogenase, dipeptidylpeptidase III, and a dipeptidase. The lung subcellular fractions were heterogeneous with cross-contamination between the heavy mitochondrial, light mitochondrial, and "microsomal" fractions. The enzyme distributions noted were similar to those found in other tissues.

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1. Otto, K., *in* "Tissue Proteinases" (A. J. Barrett and J. T. Dingle, eds.), p. 1. American Elsevier, New York (1971).
2. McDonald, J. K., Callahan, P. X., Ellis, S., and Smith, R. E., *in* "Tissue Proteinases" (A. J. Barrett and J. T. Dingle, eds.), p. 69. American Elsevier, New York (1971).
3. Rojas-Espinosa, O., Dannenberg, A. M., Murphy, P. A., Straat, P. A., Huang, P. C., and James, S. P., Infect. Immunity **8**, 1000 (1973).
4. Hook, G. E. R., Bend, J. R., Hoel, D., Fouts, J. R., and Gram, T. E., J. Pharmacol. Exp. Ther. **182**, 474 (1972).
5. Ihnen, J., and Kalnitsky, G., *in* "Intracellular Protein Catabolism, II" (V. Turk and N. Marks, eds.), p. 259. Plenum Press, New York (1977).
6. Singh, H., and Kalnitsky, G., J. Biol. Chem., in press.
7. Kalnitsky, G., Singh, H., Kuo, T., and Richerson, H. B., Fed. Proc. **36**, 1091 (1977).
8. de Duve, C., Pressman, C., Gianetto, R., Wattiaux, R., and Appelmans, F., Biochem. J. **60**, 604 (1956).
9. Canonico, P. G., and Bird, J. W. C., J. Cell Biol. **45**, 321 (1970).
10. Bosmann, H. B., and Hemsworth, B. A., Physiol. Chem. Phys. **2**, 249 (1970).
11. Garen, A., and Levinthal, C., Biochim. Biophys. Acta **38**, 470 (1960).
12. Pennington, R. J., Biochem. J. **80**, 649 (1961).
13. Nordlie, R. C., and Arion, W. J., *in* "Methods in Enzymology," Vol. IX, p. 619. Academic Press, New York (1966).
14. Chen, P. S., Toribara, T. Y., and Warner, H., Anal. Chem. **28**, 1756 (1956).
15. Iodice, A. A., Leong, V., and Weinstock, I. M., Arch. Biochem. Biophys. **117**, 477 (1966).
16. Moore, S., and Stein, W. H., J. Biol. Chem. **211**, 907 (1954).
17. Barrett, A. J., Anal. Biochem. **47**, 280 (1972).
18. McDonald, J. K., Zeitman, B. B., Reilly, T. J., and Ellis, S., J. Biol. Chem. **244**, 2693 (1969).
19. Visser, L., and Blout, E. R., Biochim. Biophys. Acta **268**, 257 (1972).
20. Bryce, G. F., and Rabin, B. R., Biochem. J. **90**, 509 (1964).
21. Kunitz, M., J. Gen. Physiol. **30**, 291 (1947).
22. Gornall, A. G., Bardawill, C. J., and David, M. M., J. Biol. Chem. **177**, 751 (1949).
23. Cooperstein, S. J., and Lazarow, A., J. Biol. Chem. **189**, 665 (1951).
24. Singh, H., and Kalnitsky, G., J. Biol. Chem. **253**, 4319 (1978).
25. Neil, M., and Horner, M. W., Biochem. J. **92**, 217 (1965).
26. Bosmann, H. B., Hagopian, A., and Eylar, E. H., Arch. Biochem. Biophys. **128**, 51 (1968).
27. Griffin, M. J., and Cox, R. P., Nature (London) **204**, 476 (1964).
28. Amar-Costesec, A., Beaufay, H., Wibo, M., Thines-Sempoux, D., Feytmans, E., Robbi, M., and Berthet, J., J. Cell Biol. **61**, 201 (1974).
29. Rademaker, W. J., "De localizatie van enige proteasne in der levercel," Kemink en zoon, Ph.D. thesis, Utrecht (1959).
30. Beaufay, H., Bendall, D. S., Baudhuin, P., Wattiaux, R., and de Duve, C., Biochem. J. **73**, 628 (1959).
31. Binkley, F., J. Biol. Chem. **236**, 1075 (1961).
32. Weiss, B., J. Biol. Chem. **205**, 193 (1953).
33. Sachar, L. A., Winter, K. K., Sicher, N., and Frankel, S., Proc. Soc. Exp. Biol. Med. **90**, 323 (1955).
34. Mallory, P. A., and Travis, J., J. Biochem. **14**, 722 (1975).

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