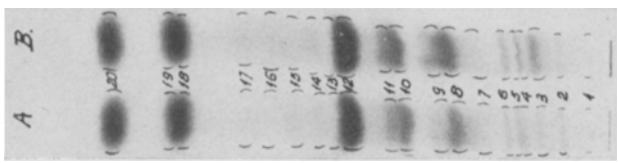
of amino acids has been made by the use of specific reactions and rechromatography. The unknown ninhydrin-reacting substances after elution with methanol were hydrolysed for 24 h (6*N* HCl, 100.0 °C).

Results. After the chromatographic analysis of ninhydrin-reacting substances in blood serum, 26 spots were identified as the free amino acids: α-alanine, α-aminobutyric acid, arginine, asparagine, aspartic acid, cysteine, cystine, citrulline, glutamic acid, glutamine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, valine. Apart from these amino acids 5 unknown ninhydrin-positive spots were detected. In all paper chromatograms only 3 from these substances were constantly found. After ninhydrin reaction they appeared as red spots. C_I spot was found under arginine, C_{II}-over arginine and C_{III}-over α-alanine (Figure).

Two other spots were not constantly present. They appeared over leucine after heating (10 min, 110 °C). After elution and hydrolysis of the 3 constantly present spots (C_I, C_{II}, C_{III}) a few amino acids were detected. In the hydrolysate of C_I spot 16 amino acids were found: α-alanine, β-alanine, α-aminobutyric acid, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, leucine, lysine, phenylalanine, serine, threonine, tyrosine, valine. From the hydrolysate of C_{II} spot 12 amino acids were obtained: α-alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, leucine, lysine, phenylalanine, proline, serine, valine. After the C_{III} spot decomposition, 14 amino acids were detected: α-alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, ornithine, proline, phenylalanine, serine, tyrosine, valine. The 2 spots from the hydrolysates were very difficult for identification. One has a Rf value higher than leucine and another smaller than cystine.



Serum free amino acids of 2 normal men (A and B) separated on Whatman No. 3 paper. Solvent system: *n*-butanol-glacial acetic acid-water (4:1:1). (1) cystine, (2) ornithine, (3) lysine, (4) histidine, (5) C_I spot, (6) arginine, (7) C_{II} spot, (8) glutamine, (9) glycine, serine, aspartic acid, (10) glutamic acid, (11) threonine, (12) α-alanine, (13) C_{III} spot, (14) proline, (15) tyrosine, (16) α-aminobutyric acid, (17) methionine, (18) valine, (19) phenylalanine, (20) leucine and isoleucine. Spots of 6 amino acids are not present because of the very small quantities.

Discussion. The free amino acid composition of blood serum is similar to that given by other authors. The unknown ninhydrin-reacting substances have also been described. No attempts, however, have been made to identify these substances. According to present experiments they seem to be peptides, because they release after hydrolysis a number of amino acids. It is difficult, however, to say whether each of the ninhydrin-positive spots contain one or more peptides. Further studies of the isolation and purification of these peptides will be undertaken.

Zusammenfassung. Mittels Dünnschicht- und Papierchromatographie wurden die ninhydrinpositiven Substanzen im Blutserum gesunder Versuchspersonen untersucht, wobei 26 freie Aminosäuren und 5 andere ninhydrinpositive Substanzen festgestellt wurden, wovon 3 neue Peptide sind.

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Relationship of Physical Factors to the Inertness of Lysosomal Hydrolases Toward One Another

Numerous soluble hydrolytic enzymes with acid pH optima are confined together in a special cytoplasmic organelle, the lysosome. Lysosomes are viewed as inert osmotic sacs delimited by an impervious lipoprotein membrane[1,2]. Lysosomes are thought to function repeatedly without altering their hydrolase composition[3]. The lysosomal enzymes appear to be unreactive toward one another both in the living cell and in situations of rapid tissue breakdown.

The unreactiveness of the lysosomal enzymes toward one another in the living cell may be related to the possibility that these enzymes are bound by loose ionic bonds to glycolipids. The lysosome and its contents may also bear an over-all negative charge, making the enzymes unreactive toward one another. Certainly the initial step of an enzyme substrate reaction would require charge differences. Histochemical staining for acid phosphatase granules has shown that these granules contain negatively charged protein glycolipid, since they are stained by basic dyes and metal ions[4,5]. Furthermore, lysosomal enzymes such as acid phosphatase and β-glucuronidase are selectively released in vitro from rat kidney, brain, and liver lysosomes by cationic molecules as diverse as acridine orange, metal ions, polyamines, an amidine, basic proteins, and phenothiazine[6,7].

A relatively high internal pH in vivo may also contribute to the non-reactivity of the lysosomal enzymes toward one another, because all of these enzymes have an acid pH optimum. These enzymes are thought to be activated by being discharged into digestive vacuoles, where an acid environment is presumed to exist. In the normal dividing cell, there is a continuous breakdown of certain intracellular proteins and other constituents. Liberated amino acids and other small molecular components are returned to the intracellular pool for new

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protein synthesis and for synthesis of other essential cellular materials.

Another factor that may be important in the in vivo non-reactivity of the lysosomal hydrolases towards other lysosomal enzymes in the lysosomes of normal dividing cells is the possibility that catheptic enzymes exist intracellularly in inactive 'ogen' forms similar to those of the trypsin-trypsinogen and chymotrypsin-chymotrypsinogen system of extracellular digestion. PRESS et al.[8] have isolated several forms of cathepsin from beef spleen. During cell death, changes in the cellular environment or a vacuolar change may bring about modifications in the catheptic structure, resulting in the activation of possible 'ogen' forms.

Binding of the lysosomal enzyme compliment to glycolipid, a negatively charged environment, a high internal pH, and possible 'ogen' forms may contribute to the relative inertness of the lysosomal enzymes to one another and their substrates in vivo. Dramatic increases in lysosomal activity occur when lysosomal membranes are broken or damaged and the lysosomal enzyme complement is spilled out into its external environment. Extracellular factors such as environmental pH and ionic strength both influence lysosomal enzyme activity. When cells die, the environment is thought to become more acidic, probably through an accumulation of short-chain fatty acids. Lysosomal enzymes are known to have their maximal activity at acid pH. Another factor contributing to lysosomal enzyme activation after the lysosomal membranes are broken or damaged may be a sudden increase in ionic strength. It is conceivable that these enzymes exist in an environment of relatively low ionic strength (i.e. bound to glycolipid), and then are activated by exposure to the external environment. Many enzymes are unstable or have little activity in regions of little or no ionic strength. The present author has found that preincubation with bovine uterine cathepsin D at 20 °C in deionized distilled water may reduce the activity of the enzyme as much as 50%. On the other hand, preincubation with this enzyme in 0.01 *M* KCl or NaCl may produce a 2- to 3-fold activation of the enzyme.

In contrast to the dramatic activation of these enzymes when exposed to their external substrates, lysosomal enzymes appear to be inert toward each other even in rapidly regressing tissues. WEBER[9] has reported a nearly 30-fold increase in acid cathepsin activity in regressing tadpole tails, with little net gain in total units of enzyme. Similarly, WOESSNER[10] has found that the total activity of uterine β-glucuronidase remained constant during late pregnancy and several days after parturition. SHAMBERGER[11] has reported striking concentrations of 6 lysosomal enzymes in regressing rat mammary tumors. Preservation of the lysosomal compliment may be important not only in regressing tissues but also in ordinary normal cells. The enigma of lysosomal enzyme preservation may be resolved by determining the turnover rates of some of the acid hydrolases in situations of rapid tissue breakdown.

Greater knowledge of tertiary protein structure and protein chemistry may also yield clues as to why lysosomal enzymes undergo turnover more slowly than do other tissue components. For example, clues regarding the inertness of the lysosomal enzymes towards one another may be gained from the knowledge of the tertiary structure of bovine alkaline pancreatic ribonuclease and papain, which, even though not lysosomal, are nonetheless hydrolytic enzymes.

X-ray diffraction studies show that a molecule of bovine alkaline pancreatic ribonuclease has inactive sulfhydryl bridges and is thus inert to sulfhydryl reagents[12]. In addition, the phosphate ion binding site, which is probably the active center, is located in a cleft of the kidney-shaped molecule. The inertness of the sulfhydryl groups and the masking of the active center, resulting in a sterically hindered molecule, may explain why lysosomal enzymes are unreactive towards one another in regressing tissues as well as in normal dividing cells. Papain has also been shown by X-ray diffraction studies to be a kidney-shaped molecule[13]. The active site of papain appears to be a groove that contains a number of substrate, binding sites, including a sulfhydryl group from cysteine[14]. Histidine may also be a part of the active site. The nature of the tertiary structure of the lysosomal remains to be elucidated. That nature, when determined may explain why these enzymes are inert toward one another.

Zusammenfassung. Die Reaktionsträgheit der Lysosomenhydrolasen gegeneinander in vivo kann aus der räumlichen Hinderung ihrer aktiven Zentren entstehen.

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Epithelial Cell Swelling During Incubation of Rat Small Intestine in vitro

The uptake of fluid by preparations of intestine incubated in vitro is well known[1,2]. The magnitude of this gut fluid uptake (GFU) is proportional to the rate of fluid transport[3,4] and, in contrast to tissues which do not perform transtissue fluid transport, is increased in the presence of metabolizable substrates and reduced by metabolic inhibitors. Although it has been suggested that the fluid taken up occupies a subepithelial extracellular compartment[2], this distribution has not been demonstrated experimentally, and no change in the extracellular space of intestine incubated in vitro could be demonstrated by conventional marker techniques[4] suggesting that the gut fluid uptake may occupy an intracellular compartment. In the present experiments the relation between uptake of fluid by intestine and epithelial cell size is examined.