aktivität gegenüber unterschiedlichen $Zn^{++}$-Konzentrationen. 1.5 ml enthalten: 80 n*M* CTP, 80 n*M* GTP, 3.3 µ*M* ATP, 0.2 µC [2-$^{14}$C]-Uridin, 7.7 µ*M* Kreatinphosphat, 200 µg Kreatinphosphokinase, 16 µ*M* $MgCl_2$, 6.2 µ*M* *Tris*, 100 µg Kalbsthymus-DNA, 0.5 ml der Enzymfraktion (entsprechend 2 mg Protein), 15–150 n*M* $ZnCl_2$, pH 7.2. Im Gegensatz zum Versuch mit intakten Aszitezellen wird hier die RNA-Biosynthese beeinflusst. $10^{-4}$ *M* $Zn^{++}$ erweist sich als wirksamste Hemmkonzentration.

Zur Klärung der Frage, inwieweit $Zn^{++}$-Ionen bereits bei niederen Vorstufen der Nukleinsäurebiosynthese eingreifen, wird die Phosphorylierung von [$^{14}$C]-markierten Nukleosiden geprüft. In der Tabelle sind die Ergebnisse zusammengestellt.

Die Biosynthese der in der Tabelle beschriebenen Nukleosidtriphosphate wird durch $10^{-4}$ *M* $Zn^{++}$ sehr schwach gehemmt. Die Untersuchungen haben gezeigt, dass $Zn^{++}$-Ionen aktiv in die Biosynthese monomerer und polymerer Nukeotide eingreifen. Die ausgeprägten Effekte des $ZnCl_2$ auf die einzelnen Parameter der Nukleinsäurebiosynthese könnten der Reaktion des $Zn^{++}$ mit den jeweiligen Polynukleotiden oder den dazu notwendigen Enzymen zugeschrieben werden. Eine Störung der Tertiärstruktur von Polynukleotiden ist durch die Spaltung der H-Brückenbindungen zwischen den Basenpaaren nach vorausgegangener Komplexbildung mit $Zn^{++}$ denkbar. Ausführliche Messungen darüber wurden unlängst durchgeführt[9–13]. Zwangsläufig würde durch diese Komplexbildung die Matrizenablesbarkeit geändert. Gleichfalls besteht die Möglichkeit, dass die monomeren Nukleotid-Zink-Komplexe die Nukleotidpolymerisation beeinflussen. Andererseits ist eine Inaktivierung der verschiedenen Polymerasen durch $Zn^{++}$-Ionen analog der hemmenden Wirkung dieser Metallionen auf die Enzymsysteme von Atmung und Glykolyse möglich[14]. Die selektive Hemmung der DNA-Biosynthese von intakten Aszitezellen könnte unter anderem auf eine Reaktion des Zinks mit SH-Gruppen des Thioredoxinsystems deuten. In diese Richtung weisen auch die Ergebnisse von Moore et al.[9], die bei Novikoff-Hepatom-Aszites-Zellfraktionen eine vollständige Hemmung der Desoxyzytidinnukleotid-Synthese durch $Zn^{++}$ beobachteten[15].

Prozentualer Anteil der Nukleosidtriphosphate an der Gesamtaktivität der säurelöslichen Fraktion nach Markierung mit [$^{14}$C]-Nukleosiden und dünnschichtchromatographischer Auftrennung der Nukleosidmono-, di- und -triphosphate % NPT-Biosynthese.

| | ATP | UTP | CTP | d-ATP | TTP | d-CTP |
|---|---|---|---|---|---|---|
| Kontrolle | 80 | 63 | 62 | 72 | 87 | 36 |
| $10^{-4}$ *M* $Zn^{++}$ | 71 | 51 | 60 | 62 | 80 | 23 |

Aszitezellsuspension 1:10 verdünnt. Je 2 ml enthalten 0.2 µC [u-$^{14}$C]-Nukleosid oder [u-$^{14}$C]-Desoxynukleosid und 200 n*M* $ZnCl_2$. Inkubation bei 37 °C für 20 min. Weitere Aufarbeitung nach Weitzel et al.[8]

*Summary.* Ascites tumour cells have been employed to study the reactivity of $Zn^{++}$ on nucleic acid biosynthesis. $10^{-4}$ *M* $Zn^{++}$ caused a selective inhibition of DNA synthesis of intact cells. The rate of RNA- and protein-biosynthesis, however, remained unchanged. The activity of DNA polymerase as well as DNA dependent RNA polymerase was strongly affected by $Zn^{++}$ in vitro.

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## Investigations on Unknown Ninhydrin-Reacting Substances in Human Blood Serum. I. Attempts at Identification of Three such Substances

In spite of numerous works on the aminoacidemia problem there are some communications about unknown ninhydrin-reacting substances in blood serum[1–4]. In recent studies, 3 ninhydrin-reacting substances were found in our laboratory[5]. The present experiments have been carried out in order to identify these substances.

*Material and methods.* Blood serum taken from 10 young healthy individuals (5 men, 5 women) was prepared according to the previous work[6]. The analysis of amino acids and other ninhydrin-reacting substances was carried out by using thin layer and paper chromatography.

Thin layer chromatography. The glass plates (18 × 25 cm) were covered with a slurry of Kieselgel G-Merck in water (1:2) at thickness of 0.25 mm. The samples of serum in quantity of 0.150–0.350 ml were applied, forming strips 20 mm in length. The plates were developed twice in the *n*-butanol-glacial acetic acid-water (4:1:1) solvent system. Spots were detected after spraying with a 0.2% ninhydrin solution in acetone.

Paper chromatography. Ascending paper chromatography on Whatman No. 3 paper was employed by using 2 solvents: (1) *n*-butanol-glacial acetic acid-water (4:1:1) and (2) methyl ethyl ketone-pyridine-water-glacial acetic acid (70:15:15:2) as previously[6]. The exact identification

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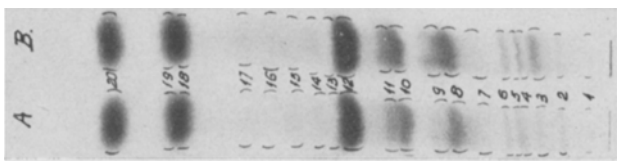
of amino acids has been made by the use of specific reactions and rechromatography. The unknown ninhydrin-reacting substances after elution with methanol were hydrolysed for 24 h (6*N* HCl, 100.0 °C).

*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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