

asparagine) a rapid enrichment of glutamine, glutamic acid, aspartic acid, and probably glutathione immediately started. A distinct but less intense enrichment of the other amino acids started rapidly, too.

Ribonucleic acid was rapidly enriched with all the other nitrogen sources, except sodium nitrate, during the first and second hour, but no greater change took place in the *desoxyribonucleic acid*.

Roine ² has earlier studied in this laboratory with other methods changes in the free amino acids during the enrichment of low-nitrogen *Torulopsis* with ammonium nitrogen being able to show enrichment of amides — glutamine and asparagine — and glutamic acid and alanine. His results have now been confirmed paperchromatographically.

By the sensitive paperchromatographic method many other free amino acids have now been shown to be present and other changes have been observed.

Virtanen *et al.* ⁶ have studied enrichment with NO₃-nitrogen finding that amino acid and amide content of the cells rises hereby much less than with ammonium nitrogen, which is in accordance with the results in this paper.

The rapid enrichment of *alanine* in these experiments must be emphasized as well as the great activity of *asparagine* as a nitrogen source, and the presence of *peptides*, which in their amino acid composition resemble more the free amino acid fraction of the yeast than the protein of the yeast.

Full details of this work are being published elsewhere.

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Notiz über die Reaktion zwischen Trialkyl-amino-silanen und Schwefelwasserstoff

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Eaborn ¹ hat gezeigt, dass Trimethyljod-silan und Silbersulfid mit einander unter Bildung von Hexamethyl-thiodisiloxan reagieren. In entsprechender Weise entstand Hexaäthyl-thiodisiloxan aus Triäthyl-jod-silan. Im folgenden werden wir eine Methode angeben, nach der Hexaalkylthiodisiloxane ohne Verwendung der schwierig zugänglichen Trialkyl-jod-silane dargestellt werden können.

Gasförmiges Schwefelwasserstoff wurde während einigen Stunden in Triäthyl-amino-silan eingeleitet. Bei der Destillation des Reaktionsgemisches wurde *teils* Triäthyl-thiosilanol vom Sdp. 158°, *teils* Hexaäthyl-thiodisiloxan vom Sdp. 128° (7 mm) erhalten. 163 g Triäthylamino-silan ergaben in dieser Weise 34,5 g Triäthyl-thiosilanol und 95,2 g Hexaäthyl-thiodisiloxan. Weiter wurden aus 89,5 g Tri-*n*-propyl-amino-silan und Schwefelwasserstoff 18,4 g Tri-*n*-propyl-thiosilanol vom Sdp. 83–84° (7 mm) und 54,3 g Hexa-*n*-propyl-thiodisiloxan vom Sdp. 168° (7 mm) erhalten.

Versuche mit Trimethyl-amino-silan konnten nicht ausgeführt werden, weil dieses Silan noch nicht dargestellt worden ist. Man hat immer statt dessen Hexamethyl-disilazin erhalten. Dieses reagierte sehr schlecht mit Schwefelwasserstoff. Dagegen ergab Trimethyl-*N*-phenyl-amino-silan mit Schwefelwasserstoff Trimethyl-thiosilanol vom Sdp. 75–76°. In einem Versuch betrug aber die Ausbeute nur 14 %.

Über die obigen Versuche und einige andere Umsetzungen zwischen Verbindungen $R_3Si-N \begin{matrix} \swarrow R' \\ \searrow R'' \end{matrix}$ (wo R Alkylgruppen und

R' und R'' Wasserstoffatome oder Alkyl-, Aryl- oder Aralkylgruppen sind) und Schwefelwasserstoff oder Merkaptanen und die dabei erhaltenen Produkte werden wir später eingehend berichten.

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Precipitation of Phosphate in the Gomori Test

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In the histochemical test for localization of alkaline phosphatase described by Gomori¹ and by Takamatsu² thin tissue slices are incubated in solutions containing glycerophosphate and calcium ions. Calcium phosphate is supposed to be precipitated at the sites of enzyme action. But calcium phosphate (in its least soluble form, *viz.* that of hydroxyapatite $\text{Ca}_5(\text{PO}_4)_3\text{OH}$) has a strong tendency to form supersaturated solutions as shown by the experiments recorded in Table 1 where $p(\text{Ca}) = -\log c_{\text{Ca}^{++}}$, $p(\text{HPO}_4) = -\log c_{\text{HPO}_4^{--}}$ and $p(\text{HAP})$ is the negative logarithm of

$$\frac{c^5_{\text{Ca}^{++}} c^3_{\text{PO}_4^{--}} c_{\text{OH}^-}}{c_{\text{H}^+} c_{\text{OH}^-}} \left(\frac{c_{\text{HPO}_4^{--}}}{c_{\text{H}^+} c_{\text{PO}_4^{--}}} \right)^3 =$$

$$= \frac{c^5_{\text{Ca}^{++}} c^3_{\text{HPO}_4^{--}}}{c^4_{\text{H}^+}}$$

$$p(\text{HAP}) = 5 p(\text{Ca}) + 3 p(\text{HPO}_4) - 4 p\text{H}$$

In the four last experiments a precipitate was formed, in expt. 2 only a slight turbidity appeared, and in expt. 1 none at all. From these and from a few other experi-

Table 1. Formation of precipitate in a solution (A) containing sodium diethylbarbiturate (0.023 M), sodium glycerophosphate (0.017 M) and the concentrations of calcium chloride and disodium phosphate given below. pH 9.4, temp. 37° C.

$p(\text{Ca})=p(\text{HPO}_4)$	$p(\text{HAP})$	$\log t_{\text{prec}}$
3.08	- 12.96	-
3.00	- 13.60	3.30
2.93	- 14.16	1.74
2.90	- 14.40	1.30
2.87	- 14.64	1.00
2.85	- 14.80	0.70

In a bifurcated vessel 5 ml solution A containing calcium chloride was rapidly mixed at 37° C with 5 ml A containing disodium phosphate to give the above solutions. A strong ray of light was thrown through the solution. t_{prec} is the time in seconds when a faint but definite Tyndall beam was observed.

ments in which the ratio $\text{Ca}^{++}/\text{HPO}_4^{--}$ was varied from 1 to 4 it is concluded that the tendency for hydroxyapatite to crystallize *spontaneously* is negligible when $p(\text{HAP}) > -13.3$ whereas the value of $p(\text{HAP})$ corresponding to solubility equilibrium is about +1. Hence there is a possibility that in the Gomori test calcium phosphate will precipitate, not at the sites of enzyme action where the concentration of phosphate is highest, but at places where there are pre-formed crystal nuclei or cell structure elements particularly favorable for adsorption, complex formation or the like.

According to Reis³ the phosphatase activity at pH 9 of human tissues may be put at 1 μg phosphorus per hour per mg wet weight on an average. Per cell of radius 10 μ it corresponds to $\gamma = 4 \cdot 10^{-17}$ moles per sec. In the pre-treatment of sections in the Gomori technique there are severe losses of phosphatase activity but we take $4 \cdot 10^{-17}$ as an upper limit for γ in a treated cell of this size. The turnover of alkaline phosphatase, *i.e.*, the number of substrate molecules converted per second