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 Gomori [1] and by Takamatsu [2] 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 $Ca_5(PO_4)_3OH$) has a strong tendency to form supersaturated solutions as shown by the experiments recorded in Table 1 where $p(Ca) = -\log c_{Ca^{++}}$, $p(HPO_4) = -\log c_{HPO_4^{--}}$ and p(HAP) is the negative logarithm of

$$\frac{c^5_{Ca^{++}}\, c^3_{PO_4^{---}}}{c_{H^+}\, c_{OH^-}} \left(\frac{c_{HPO_4^{--}}}{c_{H^+}\, c_{PO_4^{---}}}\right)^3 = \frac{c^5_{Ca^{++}}\, c^3_{HPO_4^{--}}}{c^4_{H^+}}$$

$$p(HAP) = 5\,p(Ca) + 3\,p(HPO_4) - 4pH$$

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 experiments in which the ratio Ca^{++}/HPO_4^{--} was varied from 1 to 4 it is concluded that the tendency for hydroxyapatite to crystallize *spontaneously* is negligible when p(HAP) > − 13.3 whereas the value of p(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.

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(Ca)=p(HPO_4)	p(HAP)	log t_{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.

According to Reis [3] 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

per enzyme molecule, is unknown, but we adopt the value 1000 which is very high compared with those found for other hydrolases. On this basis we find that the γ assumed corresponds to the action of $4 \cdot 10^{-17} \cdot 6 \cdot 10^{23}/1000 = 2.4 \cdot 10^4$ enzyme molecules/cell.

We assume that these molecules are packed in spherical sites of uniform size and postulate that each molecule occupies a space of $\frac{4}{3} \pi \cdot 10^{-18}$ cm^3. If the "sites" are evenly distributed throughout the cell each one of them may be regarded as being surrounded by an approximately spherical space containing no enzyme activity. When the enzymatic reaction goes on at a uniform rate in all sites the phosphate concentration will rise in accordance with a pattern which is the same in all spheres so there is no net transfer of phosphate from one sphere to another before precipitation sets in. Hence each sphere may be treated as if it were bordered by an impermeable membrane and it is possible to calculate the rate of diffusion of phosphate from the sites after a short time needed for the establishment of a quasi-steady state. The result of such a calculation is shown in Table 2 where $p(HPO_4)_{crit}$ is the negative logarithm of the concentration of secondary phosphate ion needed to bring p(HAP) to the critical value for spontaneous precipitation, p(Ca) and pH = 9.4 being given. ν is the number of sites per cell, t_{crit} the number of seconds required for the maximum concentration of phosphate to reach the critical value $(c'_o)_{crit}$. $(c'_R)_{crit}$ is the value at this time of the minimum concentration and F_R the factor by which $(c'_R)_{crit}$ exceeds the value corresponding to the solubility product. t'_s is the time required for reaching the quasi-steady state.

Table 2.

$c_{Ca++} \cdot 10^3$	$p(HPO_4)_{crit}$	ν	t_{crit}(sec)	$(c'_o)_{crit} \cdot 10^5$	$(c'_R)_{crit} \cdot 10^5$	F_R
8.4	4.63	$2.4 \cdot 10^4$	2.45	2.34	2.34	60000
»	»	10^4	2.45	2.34	2.34	
»	»	10^3	2.44	2.34	2.33	
»	»	10^2	2.41	2.34	2.30	
»	»	10	2.27	2.34	2.17	
»	»	1	1.61	2.34	1.54	39000
50	5.93	$2.4 \cdot 10^4$	0.122	0.118	0.117	59000
»	»	10^4	0.121	0.118	0.116	
»	»	10^3	0.115	0.118	0.110	
»	»	10^2	0.084	0.118	0.080	41000
»	»	10	$< t'_s$	—	—	
»	»	1	$< t'_s$	—	—	

The figures in Table 2 are only valid for a thin tissue section covered with a 10 μ thick layer of substrate solution. However, if the thickness of this layer is increased, all the values except t_{crit} will remain constant while t_{crit} will increase proportional to the thickness. It is seen that before conditions for spontaneous precipitation at the sites have become favorable there is abundant possibility for crystallization around stray crystal nuclei in the cytoplasmic material, for adsorption onto suitable cell structure elements *etc.*

In the foregoing we have assumed that if the macroscopic conditions for spontaneous precipitation are present then a sufficiently large number of crystal nuclei will be formed to ensure a defined distribu-

tion of solid particles within microscopic dimensions. But in a study on crystallization of calcium fluoride, Tovborg Jensen[4] found that a comparatively small number of solid particles (10^8 per cm^3) appear, seemingly formed from an approximately equal number of crystal nuclei by capture of ions from the solution and not by coalescence of a much larger number of nuclei. In the case of calcium fluoride there would not be one solid particle available per cell of radius $R = 10^{-3}$ cm ($10^8 \frac{4}{3} \pi R^3 = 0.4$) and even if in the case of calcium phosphate the number of nuclei per cm^3 exceeded 10^8 by several powers of ten it would be hard to imagine that finer cell structures could come out by well defined local precipitation.

The question therefore arises whether spontaneous precipitation ever occurs in the Gomori system. It seems more likely that precipitation is always induced either by pre-formed crystal nuclei or by other structure elements so that the deposits obtained are determined not only by the localization of enzymic sites but also by the localization of "precipitation centers". Recent experiments [5] confirm this view.

A more detailed treatment of the problem including aspects such as the distribution of phosphate within the sites themselves and the possibility of wandering of primary deposits of calcium phosphate toward sources of phosphate ions so as to give an increasingly correct picture, will appear in the *Acta Medica Scandinavica*.

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Aromatic Seleno- and Telluropolythionic Compounds

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This note gives a preliminary report on three new types of benzene- and *p*-toluenesulphonyl derivatives, *viz.*, selenium disulphinates, $Se(SO_2R)_2$, and thiosulphonates of divalent selenium and tellurium, $Se(S_2O_2R)_2$ and $Te(S_2O_2R)_2$. The corresponding methanethiosulphonates have been described recently [1], whereas benzene- and *p*-toluenesulphinates and -thiosulphonates of divalent sulphur, $S(SO_2R)_2$ and $S(S_2O_2R)_2$, are of older date [2, 3].

The selenium disulphinates were prepared from finely powdered, dry sodium benzene- or *p*-toluenesulphinate, suspended in dry benzene or ether, and selenium tetrachloride:

$$SeCl_4 + 3\ RSO_2Na = Se(SO_2R)_2 + RSO_2Cl + 3\ NaCl$$

The compounds also result, in equally smooth reactions, if selenium oxychloride, $SeOCl_2$, is used instead of selenium tetrachloride. Furthermore, they occur as products, beside triselenium disulphinates, when diselenium dichloride reacts with the sodium sulphinates suspended in dry ether [4].

The thiosulphonates of divalent selenium were got from diselenium dichloride and dry, powdered potassium benzene- or *p*-toluenethiosulphonate suspended in dry ether, and the thiosulphonates of divalent tellurium were obtained from the same salts, suspended in dry, ethanol-free chloroform, and tellurium tetrachloride:

$$Se_2Cl_2 + 2\ RSO_2SK = Se(S_2O_2R)_2 + Se + 2\ KCl$$