HVL	H^3-Index	Mitose-Index	$Q = \frac{H^3\text{-Index}}{\text{Mitose-Index}}$	Generationszeit (d)
Kontrolle	0,085401 M = 0,121771 0,158140	0,007268 M = 0,013047 0,018826	11,75 M = 10,08 8,40	365,92 M = 281,77 197,61
Thyreoidektomie	0,104099 M = 0,139268 0,174437	0,008533 M = 0,011535 0,014536	12,20 M = 12,10 12,00	300,20 M = 239,68 179,15
Kastration	0,349217 M = 0,293829 0,238441	0,042807 M = 0,037468 0,032130	8,16 M = 7,79 7,42	89,49 M = 110,27 131,06

Thyreoidektomie steigt der mittlere H^3-Index nur wenig, nach Kastration dagegen deutlich um den Faktor 2,5 gegenüber den Kontrollen an. Bei funktioneller Stimulation reicht also das Reservoir undifferenzierter 'Stammzellen' nicht aus, um der erhöhten Anforderung gerecht zu werden. Auch der Mitoseindex steigt an, wobei der Quotient H^3-Index/Mitoseindex annähernd konstant bleibt. Die gefundenen Quotientwerte stimmen mit Befunden an anderen Zellpopulationen gut überein[3–5]. Bei etwa gleichbleibendem Quotient muss die DNS-Synthese im HVL ganz überwiegend nachfolgend zur Mitose führen, eine endomitotische Polyploidisierung bzw. Amitosen können bestenfalls nur eine sehr untergeordnete Rolle spielen. Der Impuls für die funktionell induzierte Mitosewelle setzt dabei offenbar nicht an der Mitose selbst, sondern schon an der DNS-Reduplikationsphase an.

Unter der Voraussetzung, dass tageszeitliche Synchronisationen der verschiedenen Generationsphasen der Zelle in sehr langsam proliferierenden Organen nicht existieren[6,7] und die Länge der DNS-Synthesephase im Mittel 7,5 h beträgt, kann man eine überschlagsmässige Berechnung der Generationszeit der Parenchymzellen des HVL vornehmen. Bei den Kontrollen beträgt sie im Mittel 282 d, sie verkürzt sich 14 d nach Thyreoidektomie bzw. Kastration auf 240, bzw. 110 d. Diese – von einem «steady state» der Zellproliferation ausgehende – Berechnung bezieht sich auf das Gesamtkollektiv der HVL-Zellen. Freilich muss man damit rechnen, dass bei längeren Zeitintervallen zwischen Operation und Tötung bestimmte Teilkollektive der HVL-Zellen in eine mehr exponentielle Proliferationsphase eintreten, die zur Hyperplasie führt.

Summary. By autoradiographic studies with H^3-thymidine it is demonstrated that the percentage of labelled nuclei is very low in the anterior pituitary of normal untreated rats and of rats 14 days after thyreoidectomy. Following castration, however, a marked increase of DNA-synthesizing cells occurs as well as an increase of mitotic figures.

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Phosphoproteinphosphatase Activity in Sea Urchin Embryos

Phosphoproteinphosphatase (Phosphoprotein phosphohydrolase EC.3.1.3.16) is considered to be one of the important enzymes involved in yolk utilization[1–9]. However, in recent times its presence has been noted in a number of adult organs[10–14]. Changes in activity of the enzyme in the course of development may give a hint as to the rate at which yolk is utilized by the embryo. In this preliminary note the activity of phosphoproteinphosphatase (PPPase) has been studied during the development of *Paracentrotus lividus* and *Arbacia lixula* and compared with the known waves of rate of protein synthesis previously described[15,16].

Material and Methods. Eggs and embryos of *Paracentrotus lividus* and *Arbacia lixula* were used. Eggs were collected and washed by the usual methods[15]. Jelly coat was removed from the unfertilized and newly fertilized eggs by short exposure to acidified sea water (pH about 5.0). From the blastula stage onwards good homogenization is difficult to achieve. However, a quick washing in cold 0.22 M sucrose in $5 \cdot 10^{-4}$ M EDTA, pH 7.0, renders homogenization easier and more complete.

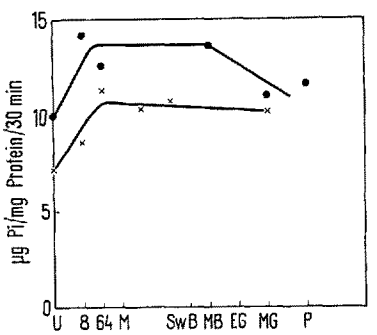
Homogenization was carried out either in 0.44 M sucrose in 10^{-3} M EDTA pH 7.0 or in 0.1 M KCl in acetate buffer 0.05 M.

A 12% solution of Hammarsten Casein (Merck Co.) prepared as described by Linderström-Lang and

DUSPIVA[17] was used as substrate. This solution was 6.13 mMolar with respect to phosphate. In some experiments, a 0.5% solution of Phosvitin (Sigma Co.) was used. This last solution was 2.83 mMolar with respect to phosphate.

Except for the experiments in which the effect of various pH and ions was assayed, the standard reaction mixture consisted of 1 ml of substrate, 1 ml of 0.1 *M* acetate buffer in 0.2 *M* KCl and 0.1 ml of homogenate or fraction thereof. Final pH of the mixture was 5.5. The reaction was carried out for 30 min at 30°C under shaking and was stopped by the addition of 0.3 ml of 60% perchloric acid (PCA); inorganic phosphate (Pi) was determined in the supernatant by the method of ALLEN[18]. Proteins were determined by the method of LOWRY et al.[19].

The echinochrome of the eggs of *Arbacia* which remains in the supernatant after precipitation with PCA and interferes with the colorimetric determination of phosphorus can be completely removed by shaking with ether.



Changes in activity of PPPase in the course of development of *Paracentrotus lividus*. Experimental conditions as described in text. Symbols: U – unfertilized eggs; 8, 64 – 8 or 64 cell stage; M – morula; SwB – swimming blastula; MB – mesenchyme blastula; EG – early gastrula; MG – mid gastrula; P – prisma.

Results. (1) It was established first that the amount of Pi released in the absence of substrate is negligible. It was further found that casein is more readily hydrolized than phosvitin, and hence the former was used in most of the present experiments.

(2) Optimum pH of activity was found to be at 5.5 for *Paracentrotus* and at 6.0 for *Arbacia*.

(3) Both $CaCl_2$ and $MgCl_2$ in the range of concentration from 5 to 50 µMole/ml proved to be inhibitory. In the eggs of *Rana*, Mg^{++} and Ca^{++} at the concentration of 100 µMole/ml completely inhibited the Pi release but were without effect at lower concentrations[9].

(4) Stimulation of activity was obtained by both NaCl and KCl at the optimum concentration of 200 µMole/ml. It must be noted, however, that this is close to the minimum concentration of these ions necessary to keep casein in solution. Therefore it is not unlikely that the requirement of these ions is mainly on the solubility of the substrate rather than on the enzyme itself. Also in the eggs of *Rana* PPPase activity was stimulated by NaCl. Here, however, the best results were obtained with 400 µMole/ml[9]. After taking into account that K^+ is by far the most prevalent ion in the sea urchin egg[20,21], KCl was used in the present experiments.

(5) The activity of the enzyme was found to be rather variable in the different batches of eggs and therefore each serial experiment was carried out on the same batch. On the average, the activities obtained are mainly of the same order as those of the frog egg[9].

As is indicated in the diagram, the activity of the enzyme increases rapidly after fertilization, reaches a maximum at about the 64-cell stage and then remains constant at least until the mesenchyme blastula. Afterwards it has been found in some cases to decline somewhat. It seems interesting to note that the rise of activity of the enzyme coincides with the first increase in the rate of protein synthesis as previously described (GIUDICE, VITTORELLI, and MONROY[15]). Whether this is due to an activation of the enzyme or to synthesis of new enzyme molecules remains to be seen[22].

Riassunto. Si è studiata l'attività fosfoproteinfosfatasica in omogenati totali di uova ed embrioni di *Paracentrotus lividus* e di *Arbacia lixula*. La reazione ha un optimum a pH acido, è stimolata da K^+ e Na^+ ed è inibita da Ca^{++} e Mg^{++}. L'attività enzimatica aumenta tra la fecondazione e lo stadio a 64 blastomeri; in seguito rimane costante fino a blastula con mesenchima; quindi generalmente decresce.

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