Erklärung der in den Tabellen verwendeten Symbole:

n Anzahl der parallelen Einzelversuche pro Serie. In jedem Einzelversuch wurden 6 × 50 Pollenkörner ausgezählt.
Prozent Keimrate, als arithmetisches Mittel aus den n Einzelversuchen bestimmt.
I,H,F, Symbole zur Bezeichnung der Wirkung: I = Indifferenz, H = Hemmung, F = Förderung.
(F,H)** Abweichung statistisch stark gesichert, $0{,}01 \geqslant P > 0{,}001$.
(F,H)*** Abweichung statistisch sehr stark gesichert, $P \leqslant 0{,}001$ *β-Indolyl-Essigsäure.*

	n	Prozent	Vergleich mit Kontrolle		
			I	II	III
Kontrolle I: Keimfähigkeit	9	21,59			
Kontrolle II: Heteroauxin 50 mg/l	8	23,42	I		
Kontrolle III: β-Karotin 1:1000	8	13,34	H***	H***	
Versuch: Heteroauxin + β-Karotin, Lösungen gemischt im Verhältnis 1:1	9	26,70	F**	F**	F***

DL-α-*Tocopherol (Vitamin E)*

	n	Prozent	Vergleich mit Kontrolle		
			I	II	III
Kontrolle I: Keimfähigkeit	6	18,39			
Kontrolle II: Vitamin E 1:2000	8	5,42	H***		
Kontrolle III: β-Karotin 1:2000	8	5,54	H***		
Versuch: Vitamin E + β-Karotin, Lösungen gemischt im Verhältnis 1:1	8	12,83	H***	F***	F***

Vitamin K (Präparat Roche 1-6722)

	n	Prozent	Vergleich mit Kontrolle		
			I	II	III
Kontrolle I: Keimfähigkeit	8	14,00			
Kontrolle II: Vitamin K 1:200	8	14,00	I		
Kontrolle III: β-Karotin 1:2000	7	7,19	H***	H***	
Versuch: Vitamin K + β-Karotin, Lösungen gemischt im Verhältnis 1:1	8	19,04	F**	F**	F***

The Pigment Granules of the Egg and Embryo of the Sea-Urchin *Paracentrotus lividus*

Previous observations have provided evidence that the carotenoid pigments of the egg of the sea-urchin *Paracentrotus lividus* are subject to changes during early development, thus suggesting that they may be involved in some morphogenetic process[1]. Since in the egg and in the cells of the embryo the pigments are located in small granules, it seemed worth while to try and get an insight into their chemical composition.

It has already been observed[2] that the carotenoids of the egg of *Paracentrotus* cannot be extracted with petroleum ether unless the eggs are first treated with alcohol. This observation has led to the suggestion that the carotenoids in the granules must be conjugated. The results of the analyses described in this paper show that the granules consist of a chromoprotein of a rather complex composition.

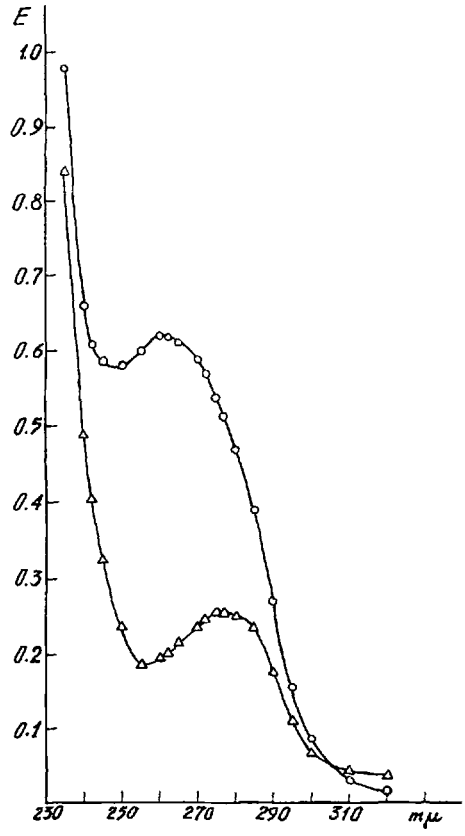
The pigment granules of eggs and embryos were isolated from homogenates in 1·5 M sucrose in 0·02 M Na-citrate. When the homogenates were centrifuged at high speed (using the high-speed attachement of the Int. Refrigerated Centrifuge) the pigment granules, together with fat, yolk granules and mitochondria, formed a thick red pellet on top of the homogenate. The pellet was easily collected with a spatula, resuspended in 1·5 M sucrose and recentrifuged. After a second washing, the pellet was taken up in 0·25 M sucrose in which the granules dissolved. A further centrifugation at high speed resulted in the separation of a clear supernatant (in order to get rid of the free fats in suspension some distilled water was layered on top of the solution before centrifugation) and a small sediment consisting of mitochondria, some unidentified granules and some pigment granules. Indeed it has constantly been noted that there is a fraction of pigment granules which does not dissolve under such conditions. All attempts to bring them into solution have so far failed. This seems to suggest that there are at least two different categories of granules in the egg. This point will be the subject of further consideration. The solution of the pigment granules in sucrose was dialysed against distilled water or buffer and submitted to analysis. All the operations so far described were carried out in a cold room at + 3°C.

The solution of the pigment granules was frozen-dried before being submitted to chemical analysis. This showed that about 30% of the dry weight consists of material extractable with 3:1 hot alcohol-ether at 70°C.

[1] A. Monroy, A. Monroy Oddo, and M. De Nicola, Exper. Cell. Res. (in press).
[2] M. De Nicola and A. Monroy Oddo (in preparation).

The extract includes carotenoids and phospholipids: indeed it contains 0·14% P. The delipidated material contains 10% N, 8·5% carbohydrates, and still 0·2% P. As no nucleic acid is present in such preparations, this residual P can be explained as being either strongly bound phospholipids or phosphoprotein-P. Paper chromatography has shown a complete assortment of amino acids.

The fact that no nucleic acid was present in our preparations seems to rule out the possibility of a contamination with mitochondria or microsomes.



Ultraviolet absorption spectrum of the chromoprotein of the pigment granules of unfertilized eggs (▵—▵) and of late blastulas (○—○) of *Paracentrotus lividus*.

The electrophoretic analysis of the granules in solution in phosphate buffer at pH 7·4 shows two main components and a third small one having mobilities of $u = -10$, $-4{\cdot}5$, and $-2{\cdot}9 \times 10^5/\mathrm{cm}^2/\mathrm{V/s}$. We cannot say at present whether the pigment is uniformly distributed in the three components, although there is some indication that the distribution may be unequal.

The fractional precipitation, using the salting out method of DERRIEN[1] (using $AmSO_4$ and estimating the amount of proteins in the supernatant from the absorption at 275 mμ), agrees with the results of the electrophoresis, showing the presence of three fractions with precipitation points at 60%, 70%, and 75% saturation of $AmSO_4$.

The same results were obtained with granules prepared from early stages of development and up to the early blastula stage. However, at about the time when migration of the primary mesenchyme takes place, the preparations appear to contain some nucleic acid. A comparison between the U.V. absorption spectrum of two preparations, one from unfertilized eggs and one from late blastula, shows this fact quite distinctly (Fig.). It is possible that the nucleic acid is a contamination, although the constancy of its quantity in different preparations (about 0·7% of nucleic acid P) may suggest that it is present as an integral part of the composition of the granules. The analysis shows that it is ribose nucleic acid, and that rules out a contamination with nuclear material. However, further analyses will clear up this important point.

Finally we might add that the chromoprotein in solution exhibits a type of reversible denaturation similar to that known for other proteins conjugated with carotenoids (Ovoverdin[1], Crustacyanin[2]): that is, the orange-red colour of our chromoprotein in solution in phosphate buffer at pH 7·4 turns to yellow when heated up to 85°C for 3–10 minutes and then, when the preparation is quickly cooled down, the colour returns to orange-red.

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Zusammenfassung

Die Pigmentkörnchen aus dem Ei von *Paracentrotus lividus* bestehen aus einem Chromoproteid. Mit Erreichen des Blastulastadiums ist in den Körnchen auch Nukleinsäure enthalten.

[1] K. G. STERN and K. SALOMON, J. Biol. Chem. *122*, 461 (1938).
[2] G. WALD *et al.*, Biol. Bull. *95*, 249 (1948).

Changes in Water Diuresis and Vasopressin Inactivation in Mice Fed on Protein Deficient Diets

It has been suggested by several authors (literature reviewed by EVERSOLE, BIRNIE, and GAUNT[1] and RALLI[2], that retention of water following liver damage is due, at least, in part to a decreased inactivation of the posterior pituitary antidiuretic hormone by damaged liver tissue. Pronounced depression of water diuresis has also been observed in animals kept on protein deficient dicts (DICKER, HELLER, and HEWER[3], DICKER[4]) and in patients suffering from hunger oedema (GOPALAN[5]). There is ample evidence to show that the liver in such deficiency conditions may not be normal. It seemed of interest therefore to investigate whether the livers of protein deficient animals inactivate antidiuretic hormone at the normal rate, and secondly whether the fatty changes frequently found in the livers of such animals have a bearing on the impairment of water diuresis or on changes in the rate of inactivation of vasopressin.

[1] W. J. EVERSOLE, J. H. BIRNIE, and R. GAUNT, Endocrinology *45*, 378 (1949).
[2] E. P. RALLI, S. H. LESLIE, G. H. STUECK, and B. LAKEN, Amer. J. Med. *11*, 157 (1951).
[3] S. E. DICKER, H. HELLER, and T. F. HEWER, Brit. J. Exper. Path. *27*, 158 (1946).
[4] S. E. DICKER, Biochem. J. *46*, 53 (1950).
[5] C. GOPALAN, Lancet *258*, 304 (1950).

[1] Y. DERRIEN, Svensk. Kem. Tid. *59*, 139 (1947).