Gerinnungszeiten der verschiedenen Plasma-Thrombin-Heparin- (bzw. -Erythrolysat-)Gemische
Plasma: Zitrat-Rinderplasma. Variable Heparinzusätze zu konstanter Thrombinmenge (Heparin «Roche»)

Heparin	Erythrolysat							Heparinmengen verschiedener Verdünnungsstufen der Erythrolysate
	Heparingehalt der Erythrolysate							
1,0 ‰	1,0 ‰	1,2 ‰	1,4 ‰	1,8 ‰	2,4 ‰	3,0 ‰	4,0 ‰	
	Gerinnungszeiten in Sekunden (Gerinnungszeit ohne Heparin: 18 s)							
26	24	25	26	26	25	26	33	0,10 mg
31	29	29	32	37	35	34	38	0,15 mg
49	36	41	39	52	46	42	45	0,20 mg
60	44	47	66	59	61	54	50	0,25 mg
>70	52	60	70	70	>70	62	59	0,30 mg
	SA-Titer der Erythrolysate							
	16	32	32	128	16	8	4	

Die Eigenschaften dieses letzteren «Stoffes» entsprechen denjenigen von Desoxyribonukleaten.)

Das Reaktionsverhältnis Heparin : Zellen : Viskositätssteigerung lässt sich in quantitativer Weise dadurch charakterisieren, dass bei konstanter Zellzahl und variierter Heparinmenge das viskositätsverleihende «Material» bestimmt wird. Zu diesem Zwecke benutzten wir eine Modifikation des ACRA-Testes[1], der bekanntlich auf mehrere hochpolymere Stoffe anwendbar ist. Wir bestimmten den Gehalt an «visköser Fraktion» in den Zellsuspensionen als diejenige Verdünnung, die im Säure-Alkohol-Test (SA-Titer) gerade noch ein zusammenhängendes Häutchen ergab. Wir stellten fest, dass die maximale Ausbeute an hochpolymerisiertem «Material» aus einer gegebenen Zellzahl an eine bestimmte Heparinmenge gebunden ist und dass dabei das Verhältnis der Erythrozyten- zur Heparinkonzentration innerhalb gewisser Grenzen ein konstantes bleibt.

Um Anhaltspunkte über die Reaktionsweise des Heparins bei diesen Vorgängen zu erhalten, prüften wir die gerinnungsverzögernde Wirkung von Erythrolysaten am Plasma-Thrombin-Gemisch im Vergleich zu reinen Heparinlösungen entsprechender Konzentration. (Wir benutzten hiezu im allgemeinen die Technik von STUDER und WINTERSTEIN[2].) In Erythrolysaten mit gewissem unterschwelligem Heparingehalt ist ein «Heparindefizit» nachweisbar (vgl. Tabelle). Auch in Erythrolysaten von höherer Heparinbeimischung ist eine Verminderung der Heparinaktivität erkennbar, jedoch nur bei den geringeren Verdünnungen des Gemisches. Erythrolysate bestimmter mittlerer Heparingehalte ergeben hingegen den entsprechenden blossen Heparindosen annähernd gleiche Gerinnungszeiten. Erythrolysate dieses Bereiches zeichnen sich ausserdem durch maximale SA-Titer, das heisst durch optimale Ausbeute an «viskösem Material» aus.

Diese Versuchsergebnisse sprechen dafür, dass, abhängig von der Heparinkonzentration, Zellbestandteile in erhöhten Quellzustand geraten und dass dabei Heparin teilweise derart involviert wird, dass dessen Aktivität im Blutgerinnungsmechanismus erniedrigt erscheint. Auffallend bleibt die unterschiedliche Beeinflussung der Heparinaktivität bei niederen, mittleren und hohen Dosen, die dafür sprechen könnte, dass entweder mehrere verschiedene Komponenten oder Reaktionen involviert sind oder dass eine bestimmte, heparinbindende Reaktion einem vom Verhältnis der Reaktionskomponenten abhängigen «Überschuss-Hemmungs-Mechanismus»[1] unterworfen ist. Diese Versuche geben somit keinen eindeutigen Aufschluss über die Reaktionsart des Heparins.

Preliminäre Versuche ergaben ein ähnliches Verhalten auch mit anderen, als Blutgerinnungshemmer bekannten Substanzen, zum Beispiel Germanin und Liquoid Roche. Weitere Untersuchungen mit analogen, aber nicht gerinnungshemmenden Stoffen sollten über die Spezifität der beschriebenen Reaktion Aufschluss geben können.

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Wissenschaftliche Laboratorien der Ciba Aktiengesellschaft, Basel, den 3. Dezember 1951.

Summary

During osmotic hemolysis in the presence of heparin there is a marked increase in the viscosity of the cell suspension, which reaches its maximum only at a certain, fixed ratio of cell quantity to heparin. The increase in viscosity is accompanied by modifications of the structure of the cell nuclei. This process appears to affect the anticoagulant activity of heparin in a certain manner.

[1] K. B. BJÖRNESJÖ und T. TEORELL, Arkiv Kemi, Min. Geol. *19A*, Nr. 34, 12 (1945).

[1] C. L. OAKLEY und G. H. WARRACK, J. Path. Bact. *63* (1), 45 (1951). - F. M. BURNET, Austr. J. exp. Biol. *26*, 71 (1948).

[2] A. STUDER und A. WINTERSTEIN, Helv. physiol. acta *9*, 6 (1951).

The Distribution of Carotenoids in the Eggs, Ovaries and Testicles of *Paracentrotus lividus*

Apart from the rôle of carotenoids as provitamins A[1] and their rôle in the visual cycle[2], very little is known of their biological function. The great accumulation of carotenoids in the gonads and eggs of a number of animals has led several workers to suppose that they may play some part in reproduction[1]. In connection with an investigation on the changes of the carotenoid pigments during the development of the sea-urchin *Paracentrotus lividus*[3] a parallel investigation has been undertaken to ascertain the quantitative ratio of the carotenoids in the

[1] T. W. GOODWIN, Biol. Rev. *25*, 391 (1950).

[2] G. WALD, The Harvey Lect. Ser. *41*, 117 (1945-46).

[3] A. MONROY, A. MONROY-ODDO, and M. DE NICOLA, Exp. Cell Res. *2*, 700 (1951).

Spectral maxima and relative percentages of the carotenoids in the extracts.

	Spectral maxima	Percentages		
		Testicles	Ovaries	Eggs
$\alpha+\beta$-Carotene	445 – 470	8·0	8·5	3·7
Echinenone	458	85·7	64·5	25·0
Xanthophyll monoester	(370)–(445)– *480*	—	9·7	27·0
Xanthophyll diester	*380* –(470)– *485*	—	2·8	5·4
Xanthophyll 1	(440)– *450* –(465)–(480)	—	14·0	10·1
Xanthophyll 2	(430)– *460* –(475)	—	—	22·0
Xanthophyll 3 (lutein)	(420)– *445* –(470)	5·9	—	—
Xanthophyll 4	(400)– *420* –(440)	0·6	—	—
Xanthophyll 5	(360)– *385 – 415 – 430*	—	2·0	—
Xanthophyll 6	(380)–(400)– *430*	—	—	5·0
Total extract: testicles	450	—	—	—
Total extract: ovaries	450	—	—	—
Total extract: eggs	475	—	—	—
Ratio xanthophylls/carotenes+ echinenone		0·005	0·37	2·4
Ratio total xanthophylls/xanthophyll esters		—	2·1	2·18
Ratio total xanthophylls/free xanthophylls		1·0	1·68	2·03

eggs, egg-free ovaries and testicles of that species. The preliminary results of this investigation will be described briefly in this article. An analysis of the carotenoids of *Paracentrotus lividus* (whole animals), followed by crystallisation of echinenone and pentaxanthine, was already accomplished by LEDERER[1]. No mention was made of differences in the distribution of the pigments in the eggs, ovaries and testicles.

The eggs were obtained by shaking the ovaries in seawater and then filtering them through cheese-cloth. After treatment with ethanol, the carotenoids could be extracted first in petrol ether (b.p. 30–50°C) and then with ether. The polar solvents alone do not allow the extraction of any pigment, thus showing that the carotenoids are present in a bound state. The extracts were washed free from alcohol with water, dried with anhydrous Na-sulphate, the solvents evaporated under a stream of N_2 and the residue redissolved in petrol ether (total extract). After repeated and careful washings to get rid of eggs and sperm, the same treatment was applied to ovaries and testicles. While the eggs after this treatment were completely colourless, the gonads still retained a pinkish red colour. On slight acidification with diluted HCl, this pigment was easily extracted with ether. Further analysis showed it to be a quinone. The total extract in petrol ether was first separated by phase partition between petrol ether and 90% methanol in free xanthophylls (alcoholic phase) and in carotenes, xanthophyll-esters and echinenone (petrol ether phase)[2].

For the chromatographic separation[3] of the free xanthophylls, $CaCO_3$ was used as adsorbent. For the separation of xanthophyll-esters, carotenes, and cheto-carotenoids (echinenone[4]) a second partition between petrol ether and 90% methanol was accomplished. The alcoholic phase contains xanthophyll-esters and monohydroxyxanthophylls and the petrol ether phase contains carotenes and echinenone. The chromatographic separation was carried out on alumina column and the purification on MgO. Free xanthophylls are retained on the latter, while carotenes and echinenone are extracted with petrol ether and xanthophyll esters with dichlorethane.

The absorption maximum of the total extract, after saponification with methanol-KOH in N_2 atmosphere undergoes a change from 475 to 450 mμ. The separated fraction of xanthophyll-esters from eggs undergoes the same change when saponified leaving free xanthophylls. Before being submitted to spectrophotometric analysis, the carotenoid fractions obtained by chromatography were re-chromatographed and whenever possible the identification was confirmed by mixed chromatography.

The quantitative data were obtained by bringing each fraction up to volume, and the relative percentages were calculated on the basis of the readings at the point of maximum absorption in the Beckmann spectrophotometer, reference being made to the total extract.

The data obtained have been summarized in the accompanying Table. α- and β-carotene have been calculated together. They are present in the eggs, egg-free ovaries and testicles. Their relative percentage is the same in testicles and ovaries, but in the eggs only about half of this quantity is found. The relative percentage of echinenone is the highest in the testicles. In the ovaries the percentage is lower by about 25% and this decrease is paralleled by a 25% increase of total xanthophylls. In the eggs echinenone is present to the extent of only 25% of total carotenoids and a further parallel, relative increase of xanthophylls occurs. According to the phase partition[1], mono- and poly-hydroxyxanthophylls are present as free xanthophylls in our material. They have been listed in the Table in the order of increasing polarity. Among these, xanthophyll 3 has been identified as lutein. The identification of the other xanthophylls is in progress. As is shown in the Table, free xanthophylls are different in the testicles, ovaries and eggs, except for one that has been found both in the egg-free ovaries and in

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[4] E. LEDERER, C. r. Acad. Sci. *201*, 300 (1935); Bull. Soc. Chim. Biol. *20*, 567 (1938). – T. W. GOODWIN and S. SRISUKH, Bioch. J. *47*, 69 (1950). – T. W. GOODWIN and M. M. TAHA, Bioch. J. *48*, 513 (1951).

[1] P. KARRER and E. JUCKER, *Carotinoide* (Birkhäuser, Basel, 1948).

the eggs. The relative percentage of free xanthophylls is highest in the eggs, then in the ovaries and lastly in the testicles. Xanthophyll-esters are present only in the ovaries and in the eggs. They have been considered as the metabolically less active form of xanthophylls and by analogy with vitamin A as a sort of storage of carotenoids[1]. The ratio of the total xanthophylls to carotenes + echinenone increases in the following order: testicles < ovaries < eggs.

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Zusammenfassung

Die relativen Mengen der Karotinoide in den Eiern, Ovarien und Hoden von *Paracentrotus lividus* sind verschieden. Qualitative Unterschiede werden in den freien Xanthophyllen gefunden.

[1] T. W. Goodwin, Biol. Rev. *25*, 391 (1950).

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Analysis of the *in vitro* Reaction between Jelly-Coat and a Cytoplasmic Component (Antifertilizin[1]) of Sea-Urchin Eggs

The ultracentrifugal isolation from eggs of *Arbacia punctulata* of a cytoplasmic component possessing the properties of Lillie's antifertilizin, has recently been reported[2]. Precipitation of the jelly-coat which surrounds the unfertilized eggs, and prevention of sperm agglutination ordinarily induced by egg-water, are properties of this component. The chemical composition of the active fraction was not investigated, attention being drawn only to its association with the pigment of the egg[2].

The reaction between jelly-coat and "antifertilizin" can be studied conveniently *in vitro*, since mixing egg extract and a solution of jelly-coat results in precipitation. Advantage was taken of this property for further study of the nature and significance of the reaction. Qualitative results of this investigation are reported here; the quantitative aspects will be discussed elsewhere.

Eggs of *Paracentrotus lividus*, *Psammechinus microtuberculatus*, *Sphaerechinus granularis* and *Arbacia lixula* were obtained by shaking the gonads in sea-water or, in the case of the latter two species, also by spontaneous shedding that follows cutting of the shell. The eggs were filtered through cheese-cloth to free them from tissue debris, and washed repeatedly in sea-water. The following operations were carried out in a cold room at 3°C. The jelly-coat solution was prepared by first treating the eggs with acid sea-water and then precipitating the jelly-coat substance by addition of 1·5 volumes of 95% alcohol[3]. After standing 30 minutes, the precipitate was centrifuged down, redissolved in about 6 volumes of distilled water and dialysed against distilled or sea-water. Egg extract was prepared by homogenising the jelly-free eggs in 0·25 *M* sucrose in a Potter-Elvehjem glass-homogenizer. The homogenate was centrifuged at 18,000 × *g* (in a refrigerated centrifuge) and the supernatant centrifuged once more with a distilled water layer on top to rid it of the free fats[1]. The final homogenate was then dialysed against distilled water. The reaction between jelly-coat solution and egg extract was done at room temperature in small test tubes.

The addition of a jelly-coat solution (in distilled or in sea-water) to an egg extract (dialysed against distilled water) of the same species results, as already mentioned, in the formation of a precipitate. Progressively increasing the amount of added jelly-coat solution yields an endpoint, beyond which further addition of jelly-coat solution no longer causes any more precipitate to appear. At this endpoint the U.V. analysis of the supernatant showed a shift in the absorption maximum to 275 mμ from 260 mμ in the original egg extract. The *N*/*P* ratio in the delipidated precipitate was about 12. While this suggests that nucleoproteins are involved in the precipitation reaction, the possibility of other substances in the egg homogenate cannot be excluded from this reaction with the jelly-coat solution; in fact, it has already been reported that lysozyme and thrombin can give a precipitation reaction with jelly-coat solutions[2]. Preliminary experiments have also shown that incubation of the egg extract with crystalline ribonuclease causes a decrease of about 1/3 in the *N* and *P* contents of the precipitate as compared with those obtained when the untreated homogenate is added to the same jelly-coat solution.

Cross-reactions between egg extracts and jelly-coat solutions of the different species have also been studied and the results are given in the accompanying Table. Jelly-coat solutions of *Arbacia*, *Sphaerechinus*, and *Psammechinus* give a strong reaction with egg extracts of all the species investigated, while jelly-coat solutions of *Paracentrotus* consistently failed to react with egg extracts of *Arbacia* and gave a weak reaction with those of *Paracentrotus* itself and of *Psammechinus*.

Jelly-coat solutions	Egg extracts			
	Arbacia	*Paracentrotus*	*Sphaerechinus*	*Psammechinus*
Arbacia	+ + +	+ + +	+ + +	+ +
Arbacia + sp.	—	—	—	—
Arbacia U.V.	—	not tried	—	—
Paracentrotus	—	+	+ + +	—
Paracentrotus + sp.	—	—	—	—
Paracentrotus U.V.	—	—	—	—
Sphaerechinus	+ + +	+ + +	+ + +	+ + +
Sphaerechinus + sp.	—	+	+	—
Sphaerechinus U.V.	—	—	—	—
Psammechinus	+ + +	+ + +	+ + +	+ + +
Psammechinus + sp.	—	—	—	—

+ sp.: jelly-coat solution treated with sperm; U.V.: jelly-coat solution irradiated with ultraviolet rays for 2 hours.

Jelly-coat solutions from all species, except *Sphaerechinus*, when "saturated" with excess of fresh sperm, proved to have lost completely their ability to react with egg extracts; the jelly of *Sphaerechinus* retains, however, some reactivity after such treatment.

For this experiment the following procedure was used. Spontaneously shed sperm was sharply centrifuged in the cold and, after removal of the supernatant sperma-

[1] The designation of this factor as "antifertilizin" seems inadequate. This term may be retained pending further studies.

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