Tabelle I

Verdünnung des Nährbodens	Exzentrische Auswanderungsförderung «*Chemotaxis*»	Zirkuläre Auswanderungsförderung					
		1:10	1:100	1:1000	1:10000	1:100000	1:1000000
Proteus vulg., lebend	+	+	+	+	+	+ –	Ø
Proteus vulg., tot	+			+	+	+	Ø
Strept. pyog., lebend	+	–	+ –	+	Ø	Ø	
Strept. pyog., tot	–	Ø	Ø	Ø	Ø		
PS-Konzentrationen		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Polysacch. aus *Proteus vulg.* . . .	+	+ –	+	+	+	+	+
Polysacch. aus *Strept. pyog.* . . .	–	Ø	Ø	Ø			

nung eine zirkuläre Auswanderungsbeschleunigung. Sie ist von der Zahl der zugesetzten Bakterien eindeutig abhängig, sie gleicht in allen Einzelheiten der in gleicher Anordnung mit Polysaccharid gram-negativer Bakterien hervorgerufenen. Ein Unterschied mag darin bestehen, dass bei hohen Keimzahlen schneller eine Hemmung der Leukozyten auftritt als bei analoger Steigerung der Polysaccharide, erklärbar durch zusätzliche Produktion toxischer Stoffe.

Gram-positive Bakterien, über die Leukozytenkulturen gleichmässig verteilt, ergeben ebenfalls eine von der Zahl der Bakterien abhängig zirkuläre Auswanderungsförderung der Leukozyten. Sie unterscheidet sich von der durch lebende gram-negative hervorgebrachten dadurch, dass die «Konzentrations-Wirkungskurve» eine andere ist. Die Auswanderungshemmung bei hohen Keimzahlen geht bei zunehmender Verdünnung in eine mässige Auswanderungsförderung über, die mit weiterer Verdünnung rasch wieder auf die Auswanderungswerte der Kontrollen zurückgeht.

Ausser den quantitativen Differenzen der Wirkung von gram-negativen und gram-positiven Keimen sind solche qualitativer Art vorhanden. Bei der exzentrischen Auswanderungsförderung, der «Chemotaxis», kommt es bei gram-positiven Bakterien leichter zu einer mehr gehäuften Ansammlung der Leukozyten, ohne die bei gram-negativen Keimen meist erhebliche Verlängerung der Auswanderungszone. Die Auswanderung unter Einfluss der gram-positiven Keime ist meist dichter als bei gram-negativen. Die Granulocyten an der Peripherie des Auswanderungsareals können eine zirkuläre wallartige Ansammlung bilden.

Die vorliegenden Versuche ergaben somit, dass lebende gram-positive und gram-negative Bakterien in ähnlicher Weise eine Steigerung der zirkulären und exzentrischen Leukozytenemigration hervorrufen. Bei gram-negativen ist diese Wirkung auf die in ihnen enthaltenen oder durch sie produzierten Polysaccharide zu beziehen, während bei gram-positiven Bakterien mit Sicherheit ein anderer Mechanismus und wahrscheinlich auch andere Stoffe für den Effekt verantwortlich sind. Es ist bemerkenswert, dass ein gleicher biologischer Effekt offenbar durch differente biochemische Mechanismen ausgelöst wird.

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Wissenschaftliche Laboratorien der CIBA Aktiengesellschaft, Basel, 14. Juli 1958.

Summary

Live gram-positive and gram-negative bacteria cause circular and 'chemotactic' eccentric promotion of leucocyte emigration in a similar manner. In the case of gram-negative bacteria this effect corresponds to that of their polysaccharides, whereas in the case of gram-positive bacteria the polysaccharides are inactive.

Thus, although gram-positive and gram-negative bacteria both have a similar effect on leucocyte emigration, the underlying mechanism of action is different.

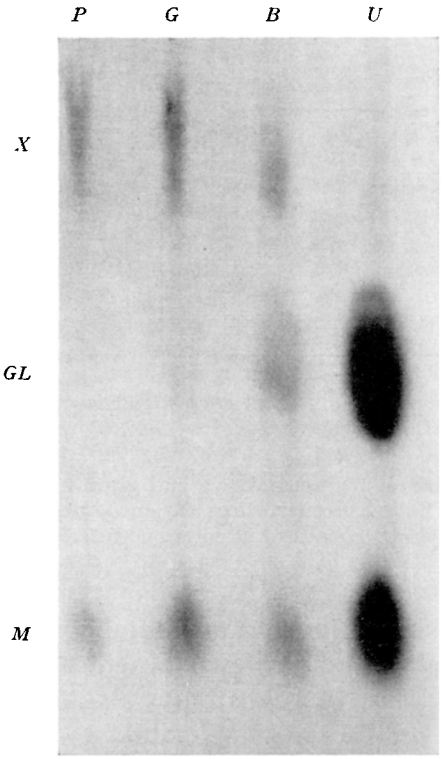
Some Observations on the Metabolism of S^{35}-Methionine During Development of the Sea Urchin Eggs

In the unfertilized egg of the sea urchin *Paracentrotus lividus* the activity of sulfur-labeled methionine has been found to accumulate mainly in the so-called nonprotein fraction (fraction soluble in 10% trichloroacetic acid, TCA). Upon fertilization (Nakano and Monroy[1]) or parthenogenetic activation (Nakano, Giudice, and Monroy)[2] the activity appears to be rapidly transferred to other cell components, among these the mitochondria. At the blastula stage an equilibrium appears to have been reached and, in fact, the activity of the TCA-soluble fraction remains almost constant until the pluteus stage. Then it appeared interesting to find out[1] in which form the labeled methionine is taken up and stored by the unfertilized egg; and[2] how it is metabolized in the course of development. Some preliminary results of this investigation will be reported here. The S^{35}-DL-methionine was administered to the eggs as previously described (Nakano and Monroy[3]). The eggs or embryos were homogenized (after removal of the jelly coat in the case of unfertilized eggs) in 10% TCA in the cold and the homogenate centrifuged at 1250 g for 10 min. The sediment was rehomogenized and, after standing overnight in the refrigerator, centrifuged as before. The extracts were pooled and shaken with several changes of ether to remove TCA. After being desalted and concentrated to a small volume, one part of the extract was hydrolyzed (with 6 N hydrochloric acid for 12 hours at 110° C). Both the hydrolyzed and nonhydrolyzed extracts were chromatographed one-dimensionally in duplicate with tertiary-butanol-formic acid-water (70:15:15) as the solvent.

For the location of the S^{35}-containing compounds the chromatogram was left in contact with an X-ray film for about 30 days. One chromatogram of each series was spread with nynhydrin, the other with potassium iodo-

[1] E. Nakano and A. Monroy, Exp. Cell Res. *14*, 236 (1958).
[2] E. Nakano, G. Giudice, and A. Monroy, Exper. *14*, 11 (1958).
[3] E. Nakano and A. Monroy, Exper. *13*, 416 (1957); Exp. Cell Res. *14*, 236 (1958).

platinate (WINEGARD *et al.*[4]) for the detection of sulfur-containing amino acids.



Radioautogram of a chromatography of the unhydrolyzed TCA-extract of unfertilized eggs (*U*), Blastulae (*B*), Gastrulae (*G*) and Plutei (*P*) of *Paracentrotus lividus* showing changes in distribution of radioactivity in the course of development. S^{35}-methionine was administered to unfertilized eggs as described in the text. *M* methionine; *Gl* glutathione; *x* spot '*x*'.

The TCA-insoluble fraction of the homogenate was extracted with alcohol-ether 3:1, once at room temperature and once in a water bath at 70° C; twice with ether-alcohol 3:1; twice with ether and finally dried. The dry powder was hydrolyzed and chromatographed as above.

The chromatogram of the TCA-soluble fraction and some control experiments soon showed that, as a result of the treatment with TCA, methionine is partially converted into methionine-sulphone. Therefore in some of the experiments the eggs were homogenized in 60% methanol (final concentration).

In the autoradiograms of non-hydrolyzed TCA or methanol extracts of unfertilized eggs two main radioactive spots are present which can be identified as methionine and glutathione. Methionine sulphone appears as a darker spot superimposed on the lower part of that of glutathione in the autoradiograms of TCA extracts.

Occasionally, one or two small radioactive spots are present close to the upper end of the glutathione.

In the autoradiograms of the hydrolyzed extracts those small spots as well as the glutathione disappear. A spot which may be identified as cystine and another one (probably cysteic acid) now become visible.

The autoradiograms of non-hydrolyzed TCA extracts of various stages of development (Figure) (blastula gastrula and pluteus) show that the spot of free methionine is present throughout development until the pluteus stage. The spot of glutathione is still evident, although considerably weaker, in extracts of blastulae; whereas in extracts of gastrulae it is barely visible and in the plutei it has disappeared entirely. In the upper part of the autoradiograms of these developmental stages a new radioactive spot (spot x) is present. Due to tailing it has a rather indefinite outline. Since upon hydrolysis it disappears it seems likely to be a peptide. The staining of the chromatograms with iodoplatinate shows a positive reaction corresponding to the position of 'spot x' not only in the developmental stages, but also in the extracts of unfertilized eggs in which no radioactivity is found in that part of the autoradiogram.

Control experiments in which unfertilized eggs were allowed to age for about 6 hours did not show any appreciable change in the above distribution of radioactivity in the TCA-soluble fraction.

It seems interesting to draw attention to the rapid uptake of the labelled sulfur atom of methionine into glutathione (probably through conversion into cysteine) which the present experiments have demonstrated in the unfertilized egg. As previously shown (NAKANO and MONROY[1]), by far the largest portion of radioactivity in the unfertilized egg is to be found in the TCA-soluble fraction and the present results now indicate that methionine and glutathione are the two major compounds in which the radioactive sulfur-atom is stored. However, in the course of development the radioactivity of glutathione disappears much more quickly than that of methionine, which in fact, is present even in the pluteus stage. No appreciable quantitative changes in glutathione have been found in the course of sea urchin development (KAVANAU[5]).

This suggests that glutathione may be considered as an intermediate through which sulfur is funnelled to the proteins of the embryo. The idea of glutathione being an intermediate in protein synthesis has been presented several times (see for example ROBERTS and BOLTON[6], HANES, HIRD and ISHERWOOD[7]). Nothing can be said at present concerning the significance of the radioactive 'spot x'.

It would be interesting if the radioactive 'spot x' present in the developmental stages and the non radioactive one present in the unfertilized eggs were the same compound. This would in fact be evidence for a peptide which is inert before fertilization and begins to exchange in the course of early development.

No clear cut results have been obtained with the protein (TCA-insoluble) fraction, the activity of the radioactive spots being too low.

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Riassunto

Si è studiato il metabolismo della metionina-S^{35} nella frazione non-proteica (TCA-solubile) nel corso dello sviluppo di *Paracentrotus lividus*.

Nell'uovo vergine la radioattività si ritrova in buona parte nel glutatione libero. Nel corso dello sviluppo l'attività nel glutatione diminuisce progressivamente mentre, contemporaneamente, si ha la marcatura di un peptide.

[4] H. M. WINEGARD, G. TOENNIES, and R. J. BLOCK, Science *108*, 506 (1948).

[5] J. L. KAVANAU, J. exp. Zool. *122*, 285 (1953).

[6] R. B. ROBERTS and E. T. BOLTON, Science *115*, 479 (1952).

[7] C. S. HANES, F. J. R. HIRD, and F. A. ISHERWOOD, Nature *166*, 288 (1950); Biochem. J. *51*, 25 (1952).

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