A NOTE ON THE BIOCHEMISTRY OF EMBRYONIC DETERMINATION IN ECHINODERMS

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Apart from the Amphibia, the echinoderms are the class of animals about which our knowledge of embryonic determination and its biochemical basis has in recent times made the most important forward strides. This is largely due to the brilliant work of the Scandinavian school. The fundamental facts of echinoderm experimental embryology are now available in a review in the English language by Hörstadius (1939), while on the biochemical side there are general accounts by Runnström (1935*a*) and particularly by Lindahl (1936).

We may mention, however, that two points of radiating influence (whether or not they should be called organizer centres remains debatable) in the echinoderm embryo, one at the animal pole, the other at the vegetal. As first proposed by Runnström, their action may best be represented in the form of two gradients, one maximal at the animal pole, the other at the vegetal. Normal development to the pluteus depends upon the "co-operation" of these two gradients, and many, if not all, of the anomalies of development may be traced to changes in their intensity. Should one centre or gradient apex be depressed, the development of the embryo veers towards the type "advocated" by the other. Since the vegetal pole forms endodermal tissues, it will, if circumstances allow it wholly to control development, bring about a "vegetalization" or "endodermization" of the embryo. In this case, presumptive ectoderm is made to form endoderm. Conversely, since the animal pole forms ectodermal tissues, it will, if given a like control of development, bring about "animalization" or "ectodermization", in which case presumptive endoderm is made to form ectoderm. When the gradients are equally powerful, the normal pluteus larva results, with its ectodermal skin, its mesenchymal spicule skeleton, and its endodermal tripartite gut. When vegetalization occurs, much material other than presumptive endoderm is deviated to form gut, which may hang out from an exo-gastrula, the ectodermal portion being reduced to a thin-walled, irregularly shaped, bag, containing deformed spicules. Conversely, when animalization occurs, no gut or skeleton ever forms at all, and the embryo becomes a thick-walled ectodermal bag provided with a very excessive complement of cilia and a hypertrophied apical tuft.

The characteristic thing about echinoderm development is that these changes

can be brought about, either by morphological methods (such as isolations and various combinations of blastomere groups, or transplantations of the very vegetal micromeres); or by chemical methods (especially ionic changes in the medium); or by a mixture of both. The classical example of a vegetalizing agent is Li', which brings about exo-gastrulation in its completest form; its action can be intensified by respiratory inhibitors such as KCN and CO. Among the most important animalizing agents are SCN' (especially when acting before fertilization), the action of which is intensified by respiratory accelerators such as pyocyanin; and lack of SO_4'' ions in the medium. These agents show many phenomena of antagonism and reversibility. They also act in a very regular and predictable way, and as already mentioned, may be combined with purely morphological methods. Such an experiment as that of Hörstadius (1936), in which animal blastomeres were isolated and then treated with Li' before transplantation, is a case in point. If such isolated animal blastomeres are vegetalized in this way, they will, on introduction into another embryo, act as powerfully in a vegetalizing direction as the micromeres themselves.

A good deal of effort has been devoted by the Scandinavian school to the biochemical explanation of the developmental deviations which chemical treatment will bring about. But a study of the literature shows nevertheless that on the whole no biochemical effects of the lithium ion comparable in magnitude to the exogastrulation effect, have been discovered. That there is a lithium-inhibitable fraction of respiration during the cleavage stages is sure, but it is not more than 25% of the total. In brei experiments (cytolysed eggs), the reduction time of methylene blue in the presence of hexose-monophosphate as donator is considerably retarded by lithium. On these grounds, Lindahl put forward the tentative hypothesis that the metabolism characteristic of the animal pole may be carbohydrate in nature. In the same way lack of SO_4'' ions also inhibits respiratory rate, but the fraction sensitive must be different because the inhibitions sum. Suggesting that the only function which sulphate ions could be likely to serve was that of detoxicating and removing aromatic waste products of protein breakdown, Lindahl framed the hypothesis that the metabolism of the vegetal pole of the egg (and hence reduced, or abolished, or rendered toxic, by animalizing agents) was of protein nature. In support of this, he succeeded in showing the existence of phenol-sulphatases in echinoderm embryos.

In the present note we shall have no more to say about the metabolism of the vegetal pole, but it occurred to us that some further light might be thrown on that of the animal pole by the use of well-known inhibitors of carbohydrate breakdown. A few experiments along this line had already been reported by Runnström (1935*b*). He exposed the unfertilized eggs of *Arbacia* to $M\ 3 \times 10^{-2}$ iodoacetate for from 2 to 6 hr., after which the small number of survivors which continued development when transferred to normal sea water showed poor development of the stomodaeum and oral arms, though the organization of the pluteus was in other respects ordinary. These effects he interpreted as very slight vegetalization, and hence confirmatory of Lindahl's hypothesis. In $M\ 3 \times 10^{-4}$ bromacetate, Tchakhotin (1938) observed

a great proliferation of primary mesenchyme, entirely filling the blastocoele cavity; this is very anomalous and cannot be regarded as evidence of vegetalization.

No experiments with *dl*-glyceraldehyde, however, existed. Mendel (1929) and Mendel *et al.* (1931) made the original discovery that this substance inhibits the glycolysis of tumour tissue, mammalian brain tissue, etc., and that the inhibition is maximal at $M\ 10^{-3}$. Much the same concentration was found to be maximally effective on the glucolysis of the chick embryo (Needham & Nowinski, 1937) and the amphibian tadpole (Nowinski, 1939). Needham & Lehmann (1937) were able to demonstrate that the inhibition is due only to the *l*-isomer, and this was confirmed by Mendel *et al.* (1938).

Many other workers (references to some of whom are given in the paper of Needham & Lehmann, 1938) have obtained similar inhibitions with glyceraldehyde on other tissues, but it has generally been believed that the processes affected are of the non-phosphorylating type, since such concentrations of *dl*-glyceraldehyde do not affect the glycogenolysis of muscle extract, for example. If, however, much stronger concentrations are used (from 10^{-2} up to molar), as in the experiments of Adler *et al.* (1937) or Boyland & Boyland (1938); or possibly if at intermediate concentrations the glyceraldehyde is present in the freshly dissolved, and hence, dimeric, form; phosphorylating glycolysis may also be inhibited.

It is well known that phloridzin also inhibits phosphorylating glycolysis (Parnas, 1937); it is maximally effective at about $M\ 0{\cdot}5 \times 10^{-2}$.

With this introduction, we may proceed to consider the experimental data we were able to procure. At Salsbury Cove, Mount Desert Island, Maine, we had an excellent supply of the sand-dollar *Echinarachnius parma*, and at Wood's Hole, Mass., we used the sea-urchin *Arbacia punctulata*. We shall omit all technical details concerning the preparation of the material, since these will be found in the recently published notes of Just (1939). The eggs were placed in the inhibitor solutions (inhibitor dissolved in sea water) as soon as it was ascertained that approximately 100% fertilization membranes had appeared. From Table I it will be seen that normal development will proceed up to $M\ 5 \times 10^{-4}$ glyceraldehyde in the case of *Echinarachnius* and Table II shows that the same is true of *Arbacia*. At $M\ 10^{-3}$ there are abnormalities of development in both cases, but the important point is that there was never the slightest trace of exo-gastrulation. Whether these abnormalities may be regarded as traces of vegetalization seems open to doubt. At $M\ 10^{-2}$ development ceases at the morula stage. Table II includes two lots of embryos which were subjected to the higher glyceraldehyde concentrations before fertilization only, developing subsequently in ordinary sea water. In this way it was possible to get some development after treatment with $M\ 10^{-3}$ and even 5×10^{-3}, but in the latter case development stopped after gastrulation and before the appearance of any arms or spicules.

The experiments with iodoacetate and phloridzin were less successful. Normal development took place in iodoacetate (see Tables I and II) in concentrations as high as $M\ 5 \times 10^{-4}$ but higher than this, even when the treatment was confined to the unfertilized eggs, no development beyond the gastrula stage was ever obtained.

Table I. *Echinarachnius parma*, *T* 14° C.

Exp. 3 and 4:

	20 hr.	32 hr.	44 hr.	54 hr.	68 hr.	
Control	Vigorous blastulae just beginning to gastrulate	Vigorous prismatic gastrulae	Young plutei with spicules forming	Normal plutei	Long-armed plutei	
Glyceraldehyde:						
10^{-2}	Morulae	—	—	—	—	no exo-gastrulae
10^{-3}	As control	As control	Prismatic gastrulae	Gastrulae with long guts but no spicules	Degenerating	
5×10^{-4}	As control	As control	As control	Young plutei	Plutei	
10^{-4}	As control	As control	As control	As control	As control	
10^{-5}	As control	As control	As control	As control	As control	
Iodoacetate:						
3×10^{-2}	Morulae	—	—	—	—	no exo-gastrulae
3×10^{-2}*	As control	No further development	—	—	—	
10^{-2}	Morulae	—	—	—	—	
10^{-3}	Morulae	—	—	—	—	
5×10^{-4}	As control	Mid-gastrulae weakly swimming	No further development	—	—	
10^{-4}	As control	Mid-gastrulae	No further development	—	—	
Phloridzin:						
10^{-2}	Morulae	Early gastrulae	Advanced gastrulae	—	—	no exo-gastrulae
5×10^{-3}	Blastulae motile but unhatched	Mid-gastrulae	No further development	—	—	
10^{-4}	As control	As control	No further development	—	—	
Lithium chloride 0·1 %	Vigorous blastulae, but spherical	Gastrulation inhibited, and many exo-gastrulae	90 % exo-gastrulae	The same	—	

* 4 hr. treatment before fertilization only.

Nevertheless, as Table II shows, this stage was often reached without the slightest sign of exo-gastrulation even at concentrations as high as *M* 3×10^{-2}, and since the maximally effective concentration in the case of tissues of vertebrates is *M* 10^{-3}, it is clear that substantial or complete inhibition of carbohydrate breakdown gives no vegetalization comparable with what we know in the case of lithium.

In the case of *Echinarachnius*, controls in 0·1 % lithium chloride were run. Practically all the embryos formed perfect exo-gastrulae.

As regards the phloridzin experiments little can be said. It is very insoluble in sea water, and though the concentrations named were attained by warming, it came out in crystalline form at the temperature necessary for normal development of the embryos. The actual concentrations must therefore have been lower than those given in the table, and the fact that normal advanced gastrulae were obtained loses much of its importance. This is particularly unfortunate since phloridzin is a specific inhibitor for phosphorylating glycolysis just as glyceraldehyde (in certain conditions) specifically inhibits non-phosphorylating glucolysis.

Summing up, it may be said, therefore, that at concentrations of *dl*-glyceralde-

Table II. *Arbacia punctulata*, *T* 23° C.

Exp. 10:

	8 hr.	18 hr.	43 hr.	
Control	Vigorous blastulae	Prismatic gastrulae	Normal plutei	
Glyceraldehyde:				
10^{-2}	As control	—	—	no exo-gastrulae
10^{-3}	As control	As control	Retarded plutei with stumpy bulbous oral arms	
5×10^{-4}	As control	As control	As control	
10^{-4}	As control	As control	As control	
10^{-5}	As control	As control	As control	
Iodoacetate:				
3×10^{-2}	Morulae	—	—	no exo-gastrulae
10^{-2}	Late morulae	—	—	
10^{-3}	As control	As control	No further development	
5×10^{-4}	As control	As control	As control	
10^{-4}	As control	As control	As control	

Exp. 11:

	12 hr.	35 hr.	53 hr.	106 hr.	
Control	Early gastrulae	Normal plutei	Advanced plutei	Advanced plutei	
Glyceraldehyde:					
5×10^{-3}*	As control	No further development	—	—	no exo-gastrulae
10^{-3}*	As control	Stunted plutei	Stunted plutei and vigorous late gastrulae with tripartite gut and no spicules	The same	
5×10^{-4}	As control	As control	As control	—	
10^{-4}	As control	As control	As control	—	
Iodoacetate:					
3×10^{-2}	Morulae	Bun-shaped blastulae beginning to gastrulate	No further development	—	no exo-gastrulae
10^{-2}	Morulae	Mid-gastrulae	—	—	
5×10^{-3}	Morulae	No further development	—	—	
10^{-3}	As control	Abnormally shaped late gastrulae, no spicules	—	—	
5×10^{-4}	As control	Abnormally shaped late gastrulae, spicules appearing	—	—	

* 6 hr. treatment before fertilization only.

hyde well within the range of maximal inhibition of carbohydrate breakdown in the tissues of vertebrates, echinoderm embryos give no sign of exo-gastrulation or marked vegetalization. The same is true of iodoacetate. Controls show that the same echinoderm material at the same time is fully capable of the marked vegetalization brought about by lithium. Hence the hypothesis of Lindahl, that the animal pole region is a centre of carbohydrate breakdown, and that vegetalization will follow if this is inhibited, acquires no further plausibility from the present results. If the reduction in length of the oral arms seen in the highest concentrations of the inhibitors is regarded as evidence of vegetalization, it must at any rate be admitted to be very feeble as compared with what will be performed by lithium. Again, it is possible that the mechanism of carbohydrate breakdown in the echinoderm embryo is quite different from those which occur elsewhere and are known to be sensitive to these inhibitors, but this is perhaps hardly very likely in view of the close

similarity between the mechanisms in yeast, bacteria, and mammalian tissues. And whether the mechanism in the sea-urchin embryo is phosphorylating or non-phosphorylating does not matter, for iodoacetate would inhibit both and glyceraldehyde the latter only.

SUMMARY

Echinoderm embryos, cultivated in *dl*-glyceraldehyde and iodoacetate, at concentrations within the range of maximal inhibition of carbohydrate breakdown as established on other systems, show no sign of exo-gastrulation or marked vegetalization. How far this affects the hypothesis of the Scandinavian school that the animal pole region in the echinoderm embryo is a centre of carbohydrate breakdown, is discussed in the text.

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