Vegetalization of the Sea-Urchin Egg by Dinitrophenol and Animalization by Trypsin and Ficin

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Herbst (1892) made the remarkable discovery that differentiation of the sea-urchin egg was shifted in a vegetal direction when lithium ions were present in the sea-water. The vegetalization implies a failure of the apical tuft to appear, a displacement of the skeleton-forming cells in the animal direction, a reduction of the ectodermal region, and a corresponding enlargement of the endoderm, which often leads to exogastrulation. Strong lithium action may lead to complete endodermization of the egg. Many other substances have later been found to cause a change of differentiation, either in a vegetal or animal direction, e.g. a partial or complete animalization by treatment of unfertilized eggs with SCN- or I-ions (also SO₄, Br, and tartrate, Lindahl, 1936). The animal and vegetal principles are considered as two opposite, antagonistic gradients (Runnström, 1928a, b) representing different types of metabolism (Lindahl, 1936).

It is of particular interest to study the effect on determination of substances which are known to interfere with metabolism in a special way. This paper will deal with the action of dinitrophenol (DNP) which acts as an inhibitor of oxidative phosphorylation, and the proteolytic enzymes trypsin and ficin.

MATERIALS AND METHODS

The experiments were made on eggs of Paracentrotus lividus. The DNP used was α-DNP and β-DNP from George T. Gurr, London, and γ-DNP from E. Merck, Darmstadt. Cleavage is immediately brought to a standstill when eggs are put into a strong solution of DNP (Clowes & Krahl, 1936, and others). Concentrated solutions are toxic. The following concentrations were found suitable: a 1/10-saturated solution of α-DNP in sea-water, a 1/4-saturated solution of β-DNP, and a 1/2- or 1/4-saturated solution of γ-DNP. The treatment as a rule took place in the 16-cell stage, before the operation when animal and vegetal halves were separated, or also immediately after the operation. In some experiments the eggs were instead put in the DNP immediately after fertilization. The results were, as we shall see, very varied, but, as far as I can judge, it did not matter

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whether the eggs were treated after fertilization, shortly before the operation, or immediately after it. The time of treatment was as a rule 2–4 hours, sometimes 6 hours, but this duration as well as longer treatments often proved disastrous, the eggs sooner or later disintegrating. A shorter treatment also often caused some mortality.

The reason for using not only whole eggs but also animal and vegetal halves is that a slight shifting of the determination in the animal or vegetal direction is difficult to register with certainty in whole eggs, whereas the animal halves are particularly sensitive in this respect. In the absence of influence from the vegetal half, the animal half as a rule shows a more animal differentiation than its prospective significance. The material of the animal half normally gives rise to about two-thirds of the ectoderm, forming an apical tuft and later pavement epithelium, ciliated band, and stomodaeeum. An isolated animal half does not gastrulate, nor does it form skeletal spicules. Furthermore, in isolated animal halves the apical tuft is as a rule more or less enlarged and neither ciliated band nor stomodaeeum are formed because of the lack of sufficient vegetal influence. In some halves the apical tuft is more or less typical. Such halves may develop a ciliated band and even a stomodaeeum. Animal halves from the same batch of eggs as a rule differentiate in a more or less uniform way, giving either the former, animal type, or the latter, vegetal type, or intermediate types.

Trypsin was used in concentrations of 0.1 or 0.05 per cent, and on halves applied after isolation, or on whole eggs after fertilization, for about 20 hours. Ficin was used in a concentration of 0.04 per cent.

VEGETALIZATION BY DINITROPHENOL

The first experiments with DNP were very puzzling. In order not to use too many watchglasses in the preliminary series I put 5 whole eggs, 5 animal, and 5 vegetal halves in the same dish. In the first series I obtained in one dish one pluteus and one larva of the prism stage type among the animal halves, whereas the other animal halves remained in the usual way as blastulae. I thought I had made some mistakes in the operations. The pluteus might have emerged from a lateral half and the prism larva could have developed from a fragment with a few vegetal cells added to the animal cells. This interpretation, however, seemed very unlikely as I have isolated thousands of animal and vegetal halves without such operational mistakes. In the next series I took particular care to check the origin of the halves. Nevertheless several plutei, prism larvae, or gastrulae with spicules appeared among the animal halves. It was therefore evident that the DNP had a strong vegetalizing effect on some individuals, whereas other animal halves, vegetal halves, and whole eggs seemed to remain unaffected. These preliminary observations called for more detailed investigations.

\(\gamma\text{-DNP}\)

I begin by describing the experiment 1950:40 which gave the clearest result.
The concentrations used were \( \frac{1}{4} \) or \( \frac{1}{2} \) of a saturated solution and are in what follows referred to merely as \( \frac{1}{4} \) or \( \frac{1}{2} \).

**Text-fig. 1.** Whole eggs treated with a \( \frac{1}{4} \)-saturated solution of \( \gamma \)-DNP in sea-water. A, B, for 2 hours, C, D, for 4 hours. (Exp. 50:40.)

Whole eggs which had been lying 2 hours in \( \frac{1}{2} \) developed into normal plutei (Text-fig. 1 A, B) and a treatment of 4 hours (C, D) also resulted in plutei, although not quite as typical as the former. We cannot, however, say that their differentiation is shifted in the animal or vegetal direction.
Vegetal halves treated for 2½ hours (Text-fig. 2b) and 4 hours (Text-fig. 2c) with 1/4 showed no difference when compared with the control halves (Text-fig. 2A).

But it is important to note that all the vegetal halves which had been lying 2½ or 4 hours in 1/2 were reduced or dead.

Examining the animal halves we find that 1/4 for 2½ hours (Text-fig. 3B) and
for 4 hours (Text-fig. 3c) caused no difference when compared with the controls (Text-fig. 3A). The picture is quite different when we compare the same controls (Text-fig. 3A) with the animal halves $\frac{1}{4}$ for $2\frac{1}{2}$ hours (Text-fig. 4A) and $\frac{1}{4}$ for 4 hours (Text-fig. 4B). In the former series all 10 halves have been transformed into plutei or larvae more or less resembling plutei with the exception of one which is still more vegetalized, forming a partial exogastrula. The 4 surviving after 4 hours in DNP (Text-fig. 4B) were gastrulae of a rather vegetal type, resembling vegetal halves.

This experiment has shown that $\frac{1}{4}$ did not have any effect, but $\frac{1}{4}$ caused a strong vegetalization of animal halves and also death in vegetal halves. At the same time no influence upon determination in whole eggs could be detected.

The conditions are, however, much more complicated than they appear in the above experiment, as will be seen from the following.

In experiment 1950:38 some also of the whole eggs became more or less vegetalized. Some differentiated as normal plutei after 1 $\frac{1}{2}$ hours in $\frac{1}{4}$ solution (Text-fig. 5 A, B), but others exogastrulated (C, D) and it seems fairly certain that endodermization has taken place in these cases. In $\frac{1}{4}$ for 3 hours plutei could still develop (Text-fig. 5G), but several larvae in $\frac{1}{4}$ for 1 $\frac{1}{2}$ hours and 3 hours closely resembled isolated vegetal halves (Text-fig. 5 E, F, H).

The animal controls produced 8 blastulae with a ciliated band and 3 with both band and stomodaeum (Text-fig. 6).
TEXT-FIG. 6. Animal halves, controls. (Exp. 50:38.)

TEXT-FIG. 7. \( \gamma \)-DNP, ½-saturated solution. Animal halves. A, treated for 1½ hours before operation; B, treated for 1½ hours after operation; C, D, treated for 3 hours before operation. (Exp. 50:38.)
In the following series 10 animal halves were treated with DNP before or after operation. The surviving halves are shown in Text-figs. 7 and 8. After 1½ hours in ½ before operation (Text-fig. 7A), only 2 did not gastrulate. One formed a small archenteron and 2 triradiate spicules, 2 produced dwarf plutei, and 3 corresponded to prism stages or a vegetal half. With ¼ for 1½ hours after operation (Text-fig. 7B) 6 developed as blastulae and 1 had a small archenteron and a spicule. It is strange that after 3 hours in ¼ before operation, the effect was less pronounced than after 1½ hours: 5 blastulae, 1 larva with small archenteron and spicules, and 1 gastrula-prism stage (Text-fig. 7c). The same treatment after operation resulted in 7 blastulae and only 1 vegetalized half, but this one formed a real pluteus (Text-fig. 7d).

In the stronger solution (¼) 1½ hours before operation the experiment resulted in 3 blastulae without skeletons, 1 blastula with a skeleton, 1 prism larva, 1 gastrula, and 1 partial exogastrula without a skeleton (Text-fig. 8A). The corresponding series treated after operation (Text-fig. 8B) showed 4 blastulae, 1 pluteus with short arms, 1 fine pluteus (skeleton found stuck to the surface), and 1 prism with large archenteron. Of the 10 halves which remained 3 hours in ½ only 3 survived. Although these had been more exposed to the DNP than those in the
other series, they differentiated completely in conformity with the controls (Text-fig. 8c).

The 12 control vegetal halves developed into more or less ovoid larvae, a typical differentiation of vegetal halves (Text-fig. 9A). A similar development was shown by the treated halves: in $\frac{1}{2}$ for 1 hour and a half hours before operation, 8 halves; 1 hour and a half hours after operation, 7 halves (Text-fig. 9B); 3 hours before operation, 4 halves; and in $\frac{1}{2}$ for 1 hour and a half hours after operation, 6 halves (Text-fig. 9C). Perhaps a faint deviation in a vegetal direction can be said to exist, but there is nothing like the strong vegetalizing effect in many animal halves. In $\frac{1}{2}$ after 3 hours the vegetal halves were reduced or died.

Some other experimental series gave similarly varied results. These may be briefly recorded. In $\frac{1}{4}$ solution for 2 hours there was the uniform result of 6 plutei, whereas in another series (with another batch of eggs) 4 hours had no effect at all. With the stronger solution ($\frac{1}{2}$) the same eggs that had given 6 plutei formed only 3 plutei out of 6 halves, and the double time, 4 hours, resulted in only 3 plutei out of 7 halves. With another batch of eggs 3 hours in $\frac{1}{2}$ had no effect on 5 halves, whereas 3 hours in the weaker solution ($\frac{1}{4}$) caused 1 out of 9 to begin gastrulation.
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\( \alpha \text{-DNP} \)

The results were similar in \( \alpha \)- and \( \beta \)-DNP. \( \alpha \)-DNP was always used in a \( \frac{1}{10} \) saturated solution. The results of treating animal halves were as follows. Only the surviving halves are recorded. The different series are separated by dashes.

2 hours: 10 blastulae.—3 blastulae, 2 plutei.—1 blastula, 2 plutei.—9 blastulae, 1 pluteus.—5 blastulae.—10 blastulae.

3 hours: 3 blastulae, 1 gastrula with skeleton, 1 pluteus.—6 blastulae, 2 gastrulae with and 2 without skeleton.

4 hours: 7 blastulae.—6 blastulae, 2 gastrulae without, 2 gastrulae with skeleton.—4 blastulae.—10 blastulae.—6 blastulae, 2 prism larvae, 1 pluteus.—10 blastulae, 1 gastrula, 3 prism or ovoid larvae.

6 hours: 2 blastulae, 1 blastula with skeleton, 1 gastrula.—8 blastulae, 1 prism larva, 3 plutei.—7 gastrulae, 1 gastrula with skeleton, 1 partial exogastrula.

\( \beta \text{-DNP} \)

A saturated solution was diluted to \( \frac{1}{4} \). The results of treating animal halves were as follows.

2 hours: 4 blastulae, 2 gastrulae with skeleton.—10 blastulae.—4 blastulae, 2 gastrulae with skeleton.
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3 hours: 3 blastulae.
4 hours: 4 blastulae,—1 gastrula with skeleton, 2 plutei,—3 blastulae.—7 blastulae, 3 gastrulae with skeleton.—9 blastulae, 1 incipient gastrulation.—2 blastulae.

CONCLUSIONS

α-, β-, and γ-DNP have the power to vegetalize animal halves in a way resembling the action of lithium ions. There are, however, some remarkable differences. We may find a slight effect resulting in an incipient gastrulation or in skeleton formation in a blastula, but also a much stronger vegetalization, from typical plutei to ovoid larvae of vegetal type and even to exogastrulae with enlarged endodermal region. The effect is, however, very variable and impossible to predict, whereas with lithium treatment the effect is often rather uniform within certain limits, when eggs from one female are treated in an identical manner. The DNP may affect one or two halves in a series but, particularly in stronger solutions, leave all the other ones unchanged. It was very rarely that all the halves in a series were vegetalized in a similar manner. It was particularly striking that several halves could show considerable vegetalization in a series immersed in a low concentration or for a short time, when stronger solutions or more prolonged treatment had no effect on eggs from the same batch.

It is also interesting to observe that vegetalization seldom occurred in whole eggs, or in vegetal halves, yet the latter showed a higher mortality than animal halves in higher concentrations or with a long treatment. This lack of a gradual increase in the vegetalizing effect of DNP on vegetal halves is in striking contrast to the action of lithium. Text-fig. 11A shows the two types which were dominant among the control vegetal halves as well as the differentiation of vegetal halves treated with 1-2, 1-9, and 2-5 per cent. Li-solution (B-D). The gradual increase of the endoderm to complete endodermization in 2-5 per cent. (D) should be compared with the negative results illustrated in Text-figs. 2 and 9; and the fact that stronger solutions or prolonged time were lethal should also be taken into account.
Motomura (1947) reports that the vegetalizing effect of lithium ions on the development of sea-urchin eggs is cancelled by α-DNP when added to sea-water containing lithium. Motomura further states that the mode of action of α-DNP is distinguished from the animalizing effect of NaSCN (Lindahl, 1936) or that of SO₄-free sea-water (Lindahl, 1935, 1936) by the fact that α-DNP does not lead to animalization when used alone, and that it insensitizes the eggs to lithium by inhibiting the cleavage.

The statement by Motomura that α-DNP has an 'animalizing' effect is astonishing in the light of our results concerning animal halves. That Motomura did not obtain any results when using α-DNP alone on whole eggs is not surprising as, according to the above experimental series, whole eggs are considerably less open to the action of DNP than animal halves, although vegetalization in some cases may be achieved (Text-fig. 5). As our results were so contradictory (Motomura claiming an 'animalizing' effect and I a vegetalizing effect), it was desirable to treat an extensive series with lithium combined with DNP.

It should be mentioned that Lindahl (1940) has found that 4-6-dinitro-o-cresol increases the action of lithium.

The experiments were made on many batches of eggs, but the eggs from any one batch were used for several concentrations and periods of treatment. The lithium sea-water contained 3 or 6 per cent. of a 3-5 per cent. solution of LiCl. The α-DNP was used in a 1/10-saturated solution in sea-water. Only whole eggs were treated. The durations in α-DNP were 4, 4 1/2, and 6 1/2 hours. The eggs did not survive a longer immersion. The lithium treatment varied between 4 hours and 24 hours. As shown in Text-fig. 12, the eggs were as a rule first put in

\[\begin{align*}
\text{4/20} & \quad \text{+++} \\
\text{6/2} & \quad \text{---} \\
\text{9/2} & \quad \text{---} \\
\text{24/2} & \quad \text{+++} \\
\text{20/2} & \quad \text{+++}
\end{align*}\]

TEXT-FIG. 12. Diagram of experiments with combined treatment of α-DNP and lithium chloride. The numerator and the broken lines indicate the duration of DNP-treatment, the denominator and the full lines the times in 3% or 6% Li-solution. O, neither Li alone nor DNP + Li had any effect. =, equal vegetalization in Li and DNP + Li. +, stronger vegetalization in presence of DNP than in Li alone. --, lesser degree of vegetalization by DNP + Li than by Li alone. The three experiments of 4/20 were performed with α-, β-, and γ-DNP respectively.
a solution containing both DNP and lithium and then after $4\frac{1}{2}$ or $6\frac{1}{2}$ hours they were transferred to sea-water, or they were, after $4\frac{1}{2}$ hours, transferred to lithium sea-water of the same concentration for continued treatment for a further $4\frac{1}{2}$ hours or 20 hours. In each experiment the following series were run simultaneously with eggs from the same batch: (1) Controls in normal sea-water. (2) A series in $\alpha$-DNP only. It can be said now that no deviation from the normal development could be detected in these. (3) A series in 3 or 6 per cent. lithium solution. (4) A series in a combined solution of $\frac{1}{10}$-saturated $\alpha$-DNP in 3 or 6 per cent. lithium. The results are summarized in Text-fig. 12 and some of the

**Text-fig. 13. Upper row, Li 6% for $4\frac{1}{2}$ hours. Lower row, $\alpha$-DNP + Li 6% for $4\frac{1}{2}$ hours.**

series are illustrated in Text-figs. 13–17. In Text-fig. 12, 0 means that neither the Li- nor the $\alpha$-DNP + Li-treatment had any effect; = indicates an equal vegetalization in both kinds of solutions; + means that the vegetalization was stronger in presence of $\alpha$-DNP than in the Li-solution; whereas - represents a lesser degree of vegetalization by $\alpha$-DNP + Li than in Li alone.

The last experiment in Text-fig. 12, 4/20, differs in two respects from the others. The eggs were first placed in sea-water containing lithium after 4 hours in DNP. Nevertheless the result was positive. Only one of these three series was treated with $\alpha$-DNP ($\frac{1}{10}$-saturated); the other two were treated with $\frac{1}{4}$-saturated $\beta$-DNP and $\frac{1}{2}$-saturated $\gamma$-DNP respectively. It is interesting to note that the three substances gave exactly similar results. The Li-controls were exogastrulae with the vegetal tip of the archenteron invaginated and with a fairly well-developed skeleton. In the DNP series the endodermization was more pronounced and the skeleton poorly developed or missing.

$4\frac{1}{2}$ hours as well as $6\frac{1}{2}$ hours was too short a time to give any Li-effect (Text-fig. 12). The larvae treated with Li-solution only form typical plutei (Text-figs. 13, 14). In 9 of the 14 series the eggs in $\alpha$-DNP + Li also showed no influence, but in 5 series there was a more or less marked vegetalization in many of the
larvae. In Text-figs. 13–17 the different types are shown in approximately the same proportions as they occurred in the cultures. We learn from Text-fig. 13, illustrating one of the 3 positive cases from the series immersed for 4½ hours, that some developed as typical or somewhat atypical plutei when others had become so strongly vegetalized as to form exogastrulae without skeleton. We here observe the same varied effect of the α-DNP as in the experiments with

Text-fig. 14. Upper row, Li 3% for 6½ hours. Lower row, α-DNP + Li 3% for 6½ hours.

Text-fig. 15. Upper row, Li 3% for 24 hours. Lower row, α-DNP for 4½ hours + Li 3% for 24 hours.

animal halves. The two positive series immersed for 6½ hours were nearly identical with those immersed for 4½ hours (Text-fig. 14). When the larvae had been exposed to lithium for 20–24 hours the vegetalization was pronounced and uniform and resulted in all series in exogastrulae of different types (Text-figs. 15, 16). The degree of vegetalization was identical in the Li-culture and in the corresponding α-DNP + Li-cultures, but only in 3 of the 12 series (Text-fig. 12, 4½/24). In the other 9 series the endodermization had gone further when α-DNP was
present, as is shown for two series in Text-figs. 15 and 16. It is striking that in these series the vegetalization in the \(\alpha\)-DNP + Li solution is much more uniform than in the above-mentioned series with shorter Li-treatment—cf. Text-figs. 15, 16 and also 13 and 14. The same holds also for the other series not illustrated here.

The experiments with a duration of \(4\frac{1}{2}\) hours in \(\alpha\)-DNP + Li followed by another \(4\frac{1}{2}\) hours in Li \((4\frac{1}{2}/9)\) show peculiar exceptions to the above results

(Text-fig. 12). In 1 out of 4 cases neither solution had any influence. In the other 3 series the Li-cultures were more or less vegetalized, whereas the \(\alpha\)-DNP + Li-larvae differentiated in a typical way (Text-fig. 17). It is hard to explain this result, which is contrary to those obtained both with shorter and longer Li-treatment. In 1 of the 3 cases the difference was very slight. The cases are therefore so few that the question arises whether the appearance of the minus cases here is incidental, or due to the particular relation between the times of action of the two solutions.

When one compares these results with those of Motomura, the following is revealed. Motomura treated eggs after fertilization with \(\alpha\)-DNP and LiCl simultaneously for 6 hours. During this period the cleavage was at a standstill. They were then transferred to sea-water. This very closely resembles our experiment

![Text-fig. 16. Upper row, Li 3% for 24 hours. Lower row, \(\alpha\)-DNP for 4\(\frac{1}{2}\) hours + Li 3% for 24 hours.](image)

![Text-fig. 17. Upper row, Li 6% for 9 hours. Lower row, \(\alpha\)-DNP for 4\(\frac{1}{2}\) hours + Li 6% for 9 hours.](image)
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It is not surprising that Motomura found no vegetalization (this lack of effect he calls animalization) as the development is arrested during the treatment and no LiCl-solution is used during the Li-sensitive period, namely during cleavage and blastula formation. Nor did I find any action of the Li-ions alone under similar circumstances (4 1/2/4 1/2 and 6 1/2/6 1/2, Text-figs. 12-14), but nevertheless the two substances combined were active (Text-figs. 12-14), a result contradictory to that of Motomura.

ANIMALIZATION BY TRYPSIN AND FICIN

It has previously been observed that the proteolytic enzymes trypsin and chymotrypsin exert a marked animalizing influence on isolated animal halves.
The same experiment has now been repeated (Text-figs. 18–20). The halves in trypsin (0·1 and 0·05 per cent.) show more decidedly animal types (see p. 328) than the control halves. Some even had the whole surface covered with long, stiff cilia (Text-fig. 18, 4/4), which otherwise never occurs in isolated animal halves, but has been observed in the isolated most animal quarter (an1-ring) (Horstadius, 1935).

**Text-FIG. 19.** Intact eggs treated by trypsin, after fertilization, for about 20 hours. Upper row 0·05%, lower row 0·1%.

**Text-FIG. 20.** Upper row, vegetal halves treated with 0·05% trypsin. Lower row, control vegetal halves.

Whole eggs and vegetal halves are also open to the action of trypsin. Text-fig. 19 shows a number of larvae from whole eggs with irregular and poor development of the skeleton and a more or less reduced archenteron. In one no invagination has taken place although a spicule has been formed. Three are completely ectodermized, resembling huge animal halves, in an early stage with enlarged apical tuft.
The vegetal halves treated with trypsin (Text-fig. 20, upper row) have a markedly smaller archenteron than the control halves (lower row), and one of them has a thick apical wall.

The proteolytic plant enzyme ficin also had an animalizing effect on isolated animal halves (Text-fig. 21). Whole larvae did not, however, show any change of differentiation.

**DISCUSSION**

It was mentioned in the introduction that the animal and the vegetal trends of development in the sea-urchin egg are considered as two opposite gradients representing different types of metabolism. From studies of the respiration in normal and Li-treated eggs Lindahl (1936) has arrived at the conclusion that the animal type is characterized by carbohydrate combustion. This hypothesis has received additional support from the fact that several substances related to carbohydrate metabolism have been found to cause an animalization of isolated animal halves, namely sodium pyruvate (Hörstadius & Strömberg, 1940), propanediol phosphate, phosphogluconic acid, and lactate (Hörstadius & Gustafson, 1947). The same result has also been achieved with intact eggs through treatment with pyruvate (Arosio et al., 1949). Ectodermal development, i.e. the animal metabolic type, favours the development and activity of mitochondria including protein synthesis (Gustafson & Hasselberg, 1951; Gustafson & Lenicque, 1952; Gustafson, 1952).

Li-ions cause vegetalization (Herbst, 1892). In extreme cases they may convert the whole egg to endoderm. Lack of SO₃⁻-ions results in an animalization (Herbst,
because of an inhibition of the vegetal processes. According to Lindahl (1936), this is due to toxic waste products of aromatic amino acids which normally, in presence of SO$_4$-ions, are detoxicated by phenolsulphatases. The endomesodermal development, i.e. the vegetal metabolic type, may therefore be characterized by a protein combustion and a sulphate esterification.

It is known that DNP prevents the use of energy provided by respiration and glycolysis, and it has been suggested that it does so by inhibiting the formation of high-energy phosphate bonds (Lardy & Elvehjem, 1945; McElroy, 1947). This hypothesis was supported by Loomis & Lipmann (1948), who showed that low concentrations of DNP reversibly uncouple the phosphorylation associated with the oxidation of glutamate. The depression of oxidative phosphorylation by DNP was confirmed by Judah & Williams-Ashman (1950) using a number of substrates.

DNP has in this paper been shown to cause a strong vegetalization in many cases, transforming animal halves to plutei, even of vegetal type. This implies a morphological counterpart to the action of lithium on animal halves (von Ubisch, 1925; Hörstadius, 1936). The primary point of attack of lithium in the metabolism has long been unknown. The decrease of respiration in the presence of lithium is important. In normal development there are three periods of increasing respiration, the curve showing the shape of an S, a straight line, and an exponential rise. Gray (1927) and Lindahl (1936, 1939) furthermore discern a constant and an increasing part of the respiration. In the first period lithium probably inhibits the increasing but not the constant part; in the following periods it probably also inhibits the constant part, acting in another way (Lindahl, 1939). The vegetalizing effect of lithium is strengthened by lowering respiration through CO + O$_2$, KCN, or partial anaerobiosis (Runnström, 1928b, 1933; Lindahl, 1936, 1940). The importance of respiration for the animal trend of development is illustrated by the fact that animalizing agents such as SCN-, I-, and Br-ions are only efficient in presence of O$_2$ (Lindahl, 1936), and that pyocyanine intensifies their effect (Runnström & Thörnblom, 1938). Lithium is also counteracted by K-ions, with regard to both morphology (Runnström, 1928) and respiration (Lindahl, 1936). Li$^+$ may competitively displace K$^+$ from an enzyme (Lindahl, 1936). It has been suggested that K$^+$ is essential for the phosphate transfer from 2-phospho-pyruvate to ADP (Kachmar & Boyer, 1951). It has actually been shown that an addition of lithium ions to a culture of sea-urchin eggs causes an accumulation of inorganic pyrophosphate during cleavage, i.e. during the period when Li$^+$ promotes vegetalization (Lindahl & Kiessling, 1951). Lithium evidently, therefore, brings about abnormalities in the phosphate metabolism.

Glutamine causes animalization in animal halves (Hörstadius & Strömberg, 1940; Hörstadius, unpublished). Gustafson & Hasselberg (1951) found that Li treatment during intermediate cleavage stages strongly retards the normal increment of glutaminase activity at the onset of gastrulation. They point out
that the energy released in carbohydrate breakdown is transferred to phosphate bonds which is in accordance with the increase of ATP-turnover at the mesenchyme-blastula stage. The phosphate bond energy would be used inter alia for glutamine synthesis which is known to consume ATP (for references see Gustafson & Hasselberg, 1951). This gives us another case where lithium may interfere with phosphorylation, inhibiting a synthesis, and thereby causing a vegetalization.

We have seen that several investigators have brought forward evidence supporting the idea that lithium interferes with phosphorylation, and that Lindahl & Kiessling (1951) have proved a real disturbance of phosphate metabolism by lithium. Similar morphogenetic effects to those of lithium may be achieved by DNP, which is known to inhibit the energy transfer although its real point of attack in the cycle seems to be still obscure. This result gives additional support to the results and hypotheses concerning the action of lithium.

It is of great interest in this connexion that a strong vegetalization including exogastrulation has been obtained by sodium azide (Child, 1948, 1953) which attacks the cytochrome system.

The action of DNP is peculiar in several respects. Its effect may be as strong as that of lithium, transforming even animal halves to plutei of vegetal type. On the other hand, its action is very selective, many larvae in an experiment being altogether unaffected. Furthermore, entire larvae only rarely showed any vegetalization, and vegetal halves either developed like the controls or they disintegrated. On the contrary, various degrees of vegetalization are obtained when vegetal halves are treated with lithium (Text-fig. 11).

Combination of DNP and Li often gave a stronger vegetalization than either of these agents alone. In those series (Text-figs. 12–17) in which whole eggs were treated, the DNP alone had no effect. In some series with DNP + Li the larvae did not deviate from the controls (0 in Text-fig. 12). In 3 series the degree of vegetalization was the same in DNP + Li as in Li alone (= in Text-fig. 12). In the majority of cases where differences between the two existed the larvae in DNP + Li were more vegetalized than the pure Li-larvae (+ in Text-fig. 12, Text-figs. 13–16). Only 3 series gave the opposite result (− in Text-fig. 12, Text-fig. 17). Although the comparatively short early treatment with DNP alone is negative, it evidently prepares the ground for, or strengthens, the action of lithium. This perhaps is another indication that both substances are interfering with the same kind of processes.

The statement that trypsin and chymotrypsin exert an animalizing influence on isolated animal halves (Hörstadius, 1949) has now been confirmed and a strong effect also demonstrated on intact eggs (Text-fig. 19). Moore (1952 a, b) also claims the same result on whole eggs of Strongylocentrotus droebachiensis when exposed to trypsin before fertilization. Subsequent treatment by lithium neutralized the effect. But Moore's photographs give the impression more of inhibited gastrulation than of real animalization.
The statement that the plant enzyme ficin also has an animalizing action on animal halves (Text-fig. 21) invites the question whether all kinds of proteolytic enzymes have a similar effect. Further investigations are needed.

It is not easy to imagine how the proteolytic enzymes work in this case. A complete permeation so that molecules interfere with protein metabolism in the interior is not very likely. Some authors have found that the hyaline membrane is digested by trypsin but cleavage and gastrulation may proceed, whereas other authors claim that the hyaline layer is more or less resistant. The result evidently depends on differences in experimental conditions (literature in Bohus Jensen, 1953). One assumption to explain the animalizing action might be that trypsin changes the permeability of the surface layer so that substances may pass in or out in an abnormal way. Another possible explanation is that the enzyme attacks a superficial substance which is of special importance for the vegetal trend of development. A third explanation is that some proteolytic degradation products from the surface may penetrate to the interior.

**SUMMARY**

1. α-, β-, and γ-dinitrophenol have the power to vegetalize sea-urchin eggs, particularly animal halves, in a manner resembling the action of lithium ions, but with some differences. The effect is very varied, often only a few of the treated eggs or animal halves becoming vegetalized. Vegetal halves either developed like the controls or they disintegrated; whereas different concentrations of LiCl caused different degrees of vegetalization of vegetal halves.

2. Combination of DNP and lithium often causes a stronger vegetalization than either of these agents alone.

3. The possibility that lithium and DNP attack metabolism at the same point is discussed.

4. Trypsin causes a strong animalization of whole larvae as well as of animal and vegetal halves. An animalization of animal halves was achieved also with ficin.

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AND SEA-URCHIN DEVELOPMENT

REFERENCES


