Epithelial cells of the paraphysis	*Epithelial cells of the choroid plexuses*
1 Mostly cubic or low cylindric.	1 Mostly flat or low cubic.
2 Nuclei mostly ovoid to oval with long axis perpendicular to the free surface of the cell.	2 Nuclei mostly oval with their long axis parallel to the free surface of the cell.
3 Relatively much nuclear substance in cell.	3 Relatively much more cytoplasm in cell.
4 Continuous thin cuticula lining the free border. Broken up during secreting phase.	4 Relatively high striated cuticular membrane, never broken up.
5 No cilia.	5 Cilia in bundles from the parts of some cells.
6 Basement membrane in immediate contact with the endothelium of venous sinusoids.	6 Basement membrane especially in younger stages much less in contact with endothelium of choroidal vessels but more with underlying mesenchyme.
7 No attachement of particulated matter injected.	7 Strong attachment of particulated matter especially at the cilia.
8 Cells have secretory function.	8 Cells have certainly resorbing function. Secretory function could not be demonstrated in the experiment described.

secreting activity of the cells could also be detected although in a much less conspicious way. Here the secretion happens merely merocrine and eccrine.

It stands to reason that these experiments do not give us a conclusive idea as to the quality of the substance evidently secreted by the paraphysis. It may be possible that in the experiment described some cerebrospinal fluid is lost and that the paraphysis tends to secrete more liquor than it does normally making up for the loss or for the eventual disturbed chemical balance caused by the suspension injected. It may also be that the paraphysis secretes only more special chemical substances. But, as it is, this investigation points strongly in the direction that in Urodela the paraphysis is more concerned in the production of the spinocerebral fluid or of its constituents whereas the plexuses may have primarily if not exclusively an absorbing function. Summarizing, some differences between the epithelium of the paraphysis cerebri in Urodela and that of the choroid plexuses are computed see above.

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Anatomisch-Embryologisch Laboratorium der Rijksuniversiteit te Groningen, November 24, 1948.

Zusammenfassung

Die Paraphysis cerebri ist ein zusammengesetztes tubulöses Organ, das sich in den Ventriculus impar telencephali, den vorderen Teil des 3. Ventrikels, eröffnet. Die Tubuli bestehen aus einem einschichtigen hauptsächlich kubischen bis zylindrischen Epithel auf einer Basalmembran, die mit wenigen Ausnahmen unmittelbar an die Wand von venösen Sinusoiden grenzt. Nach intrazerebralen Injektionen durch das Dach des 4. Ventrikels von *Amblystoma mexicanum* mit einer 5%-Suspension von Tusche in isotonischer Ringer-Lösung zeigen sich in den Schnitten gar keine Tuscheteilchen in den paraphysären Tubuli, während diese auf und in dem Ependym und vor allem dem Epithel aller Plexus chorioidei in großer Menge sichtbar sind. Die schwarzen Teilchen werden offenbar in den Plexus resorbiert. Weiter wird eine Sekretion in den paraphysären Tubuli in der Richtung des Lumens, also nach dem Ventrikel zu, beschrieben und abgebildet. Aus dieser vorläufigen Mitteilung geht also hervor, daß die Paraphysis von Urodelen eine zusammengesetzte tubulöse extern sezernierende Drüse ist, die einen oder mehrere, wenn nicht alle Bestandteile des Liquor cerebrospinalis produziert, und daß dem Epithel der Plexus jedenfalls eine resorbierende Funktion zukommt. Eine Tabelle zeigt einige Unterschiede zwischen dem Epithel der paraphysären Tubuli und demjenigen der Plexus chorioidei.

The Effect of Nitrogen-Mustard on Sea-Urchin Eggs

It is well known that nitrogen-mustard compounds cause a delay or inhibition of mitotic divisions in yeast[1], *Tradescantia*[2], and *Triton embryos*[3]; an effect on cleavage of sea-urchin eggs has also been reported[4]. The present work was carried on the fertilized and unfertilized eggs of two species of sea-urchin (*Arbacia lixula* and *Sphærechinus granularis*) at the Zoological Station Naples, in August and September, 1948, while the senior author was on a UNESCO fellowship.

Eggs were treated with hydrochloride salt of methyl (dichloroethyl)amine. No cleavage occured in the first hour after insemination, and from then observations were made at intervals of 10 minutes to 1 hour up to 8 to 10 hours.

The results are expressed graphically as number of divisions plotted against time after insemination. Divisions 1, 2, 3, 4, 5, and 6 represent the number of blastomeres 2, 4, 8, 16, 32, and 64 respectively. Each point in the graphs represents the average number of blastomere per sample at a given time.

Eggs treated with 0·001%—0·005% of the substance for 10 minutes developed normally up to the 4th day, forming normal plutei. There was only slight decrease in rate of cleavage as compared with the controls (Fig. 1). Longer treatment at these concentrations resulted in abnormal gastrulæ or failure of gastrulation. A concentration of 0·01% for 10-minutes treatment delayed cleavage more considerably, but the eggs reached the 6th division forming normal swimming blastulæ. They however, only reached the beginning of invagination and cytolysed in a further 20 hours. Longer

[1] V. E. KINSEY and W. M. GRANT, J. cell. and comp. Physiol. *29*, 51 (1947).

[2] P. C. KOLLER, M. Y. ANSARI, and J. M. ROBSON (1943), quoted in: J. Exp. Zool. *103*, 1 (1946).

[3] R. GILLETTE and D. BODENSTEIN, J. Exp. Zool. *103*, 1 (1946).

[4] R. K. CANON et al. (1943, 1944), quoted in: J. Exp. Zool. *103*, 1 (1946).

treatment prevented the formation of normal blastulæ. Concentrations above 0·01% delayed cleavage to a considerable extent and the eggs never reached 64 cells. Some eggs showed irregular cleavage planes even at the first division. The degree of such irregularity and the

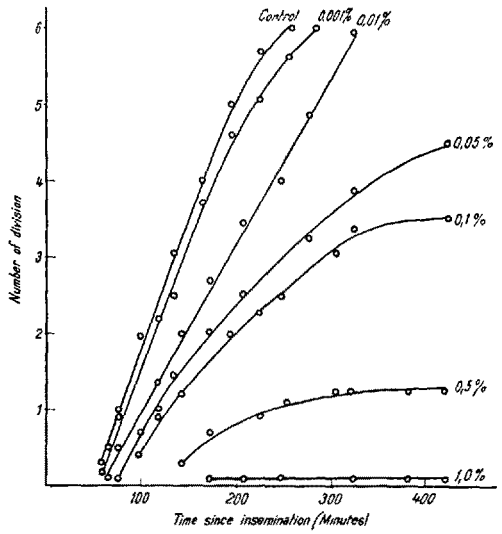

Fig. 1. – Retardation of cleavage by different concentrations of nitrogen-mustard at room temperature.

maximum number of blastomeres they could form depended on the concentration of the substance and the length of the treatment. Using 0·05% for 10 minutes, about half of the eggs ceased to develop further than the 4th division. With 0·1%, they could hardly complete the 4th division. At 0·5%, most of the eggs stopped developing after first irregular cleavage, and at 1·0% only a small minority of eggs began to divide. In the last two cases, the eggs soon became coagulated and cytolysed in about 24 hours.

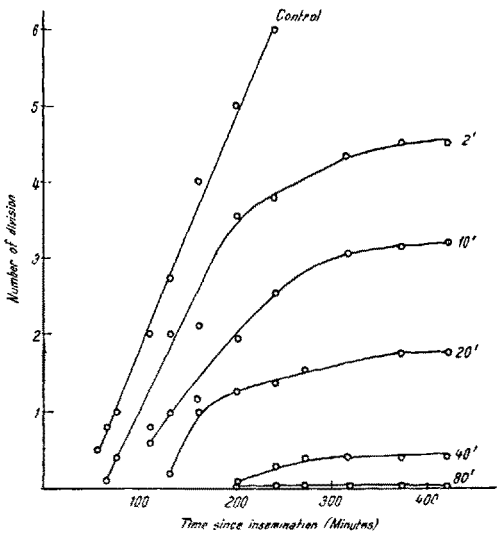

Fig. 2. Retardation of cleavage by concentration of 0·1% after different length of time of treatment following insemination at room temperature.

Other series of experiments were run, employing concentrations from 0·01 to 0·1%, but varying lenghts of treatment. The longer time of treatment, the stronger is the effect and the earlier the eggs become incapable of further development. A typical case is represented in Fig. 2, which gives the result of a series of experiments with an 0·1% solution. The eggs were treated after insemination at intervals from 2 to 80 minutes. The graphs show the results.

The effect of nitrogen mustard on the different stages of development was then studied. Two types of experiments were made. First, three sets of eggs, namely, unfertilized, two minutes after insemination, and 20 minutes after insemination were submitted to varying concentrations for fixed times. Secondly, using only fertilized eggs and one-minute treatment with 0·1% solution, the effects at varying times after insemination were studied up to 90 minutes.

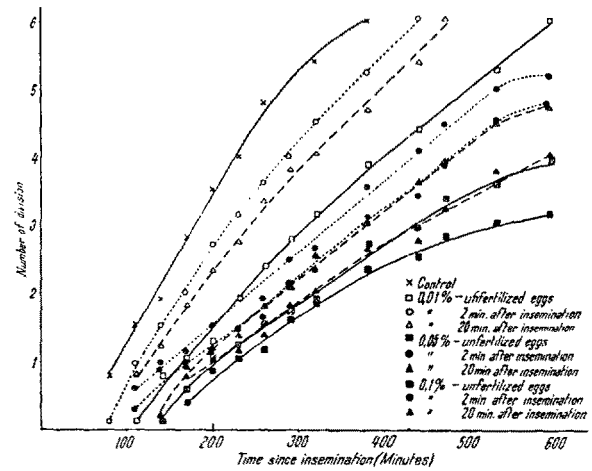

Fig. 3. – Differential susceptibility to varying concentrations of nitrogen-mustard at different stages of development at 18°C.

Fig. 3 describes the results of two experiments of the first type, using three different concentrations. The length of treatment was 10 minutes in each case. The retardation effect is most pronounced in the unfertilized eggs, suggesting a higher susceptibility in this stage. Eggs treated 2 minutes after insemination appear to be relatively more sensitive as compared to those treated 20 minutes after insemination, though the difference is not significant especially with respect to the late cleavage divisions. Observations on the development subsequent to the morula stage are also in agreement with the above. At concentration 0·01% while the fertilized eggs formed free-swimming blastulæ similar to the controls, although with a delay of about 40 minutes, the unfertilized eggs succeded to hatch in due course of time, but remained as massive ball of cells which soon cytolysed. The fertilized eggs did, however, differ from the control in that if treated 2 minutes or 20 minutes after insemination were not capable of completing gastrulation. At higher concentrations, namely, 0·05% and 0·1%, where cleavage division stopped before 64-cell stage, subsequent development likewise differed between the unfertilized and fertilized eggs. In the former the irregular massive ball of cells started cytolysis before they showed any sign of hatching, whereas in the fertilized eggs most of them died during hatching.

The results of the second series of experiments with one-minute treatment are presented in Table I, in which each value represents an average of readings taken from two experiments. The intensity of the effect decreases as interval between insemination and treatment becomes longer. But even as late as 90 minutes after insemination, one-minute treatment in 0·1% solution proved to be effective not only in delaying the rate of cleavage beyond the first one (which most of the eggs

Table I

Development of eggs treated with 0·1% at various times after insemination for one-minute interval (at 18°C).

The figures represent the average number of blastomeres.

Observation time (minutes after insemination)	Time of treatment (minutes after insemination)												
	0	2	4	6	8	10	12	15	20	30	60	90	control
80												1·1	1·1
90												1·5	1·5
100			1·2	1·2	1·2	1·2	1·3	1·3	1·3	1·4	1·6	1·9	2·0
130	1·2	1·6	1·9	1·9	1·9	1·9	1·7	1·8	1·8	2·1	2·1	2·5	2·7
160	1·5	2·0	2·2	2·2	2·2	2·3	2·3	2·5	2·3	2·5	2·8	3·6	3·8
180	1·9	2·6	2·8	2·8	2·8	2·9	3·1	3·5	3·0	3·3	3·8	4·6	6·6
240	3·2	3·8	4·1	4·1	4·2	4·2	5·4	5·8	5·2	5·5	7·1	9·2	15·4
300	4·8	5·8	6·8	6·9	6·8	7·3	8·5	8·9	8·2	9·0	11·9	12·9	28·0
360	6·7	8·6	11·3	11·1	11·4	11·1	12·7	14·9	12·4	13·8	17·1	16·5	31·0
420	7·7	10·0	13·0	14·4	13·5	14·4	14·2	15·3	13·4	14·4	19·0	19·7	64·0
480	9·7	12·5	14·1	14·9	14·9	14·9	15·1	15·8	14·4	14·9	21·0	22·4	morula
540	11·6	13·6	15·5	15·8	15·3	16·1	16·4	16·8	15·1	16·3	24·9	26·8	morula

had completed before treatment) but also in inhibiting formation of normal blastulæ.

When the eggs were treated 2 minutes after insemination, the effect was weaker than the instantaneous treatment, but definitely stronger than the treatments at any later period. Treatments that took place from 4 to 30 minutes after insemination did not show much difference. Apparently in the fertilized eggs there is not a specific sensitive period in respect to the action of the substance.

The eggs treated soon after insemination were most seriously affected in the whole series. They also differ from the eggs treated later in two other respects: first, less than half of the eggs elevated the fertilization membrane, and those not showing it were incapable of further development; and, second, about one-fifth of the fertilized eggs showed polyspermy. Possibly, the normal mechanism of the formation of fertilization membrane may be partly impaired resulting in total failure of the fertilization process, or weakened to such an extent as to permit the entrance of more than one sperm.

The rate at which the mustard interferes with the development of the sea-urchin eggs is apparently related to both the concentration and the length of treatment. In this respect the behaviour resembles very much to the effect of the ultra-violet rays reported by GIESE[1]. It is entirely different from another group of substances, such as we also were able to test, chloropicrin and chloracetophenone.

With both of them, concentrations above 0·002% or treatment of 0·001% for more than 10 minutes are effective in killing *Arbacia* fertilized eggs immediately. But in concentration 0·001 for 10 minutes or less, the eggs developed normally without even showing significant retardation of cleavage divisions. These cases rather demonstrate an all or non-effect.

The effect of nitrogen-mustard on sea-urchin cleavage also shows that there is no latent period between the exposure of the egg and the effect produced. This can best be demonstrated by the fact that eggs, when treated 90 minutes after insemination, at which time most of the eggs have completed the first cleavage, have their second division immediately affected. This is also true for yeast[2].

[1] A. C. GIESE, Biol. Bull. *74*, 330 (1938).
[2] V. E. KINSEY and W. M. GRANT, l. c.

That unfertilized eggs and eggs treated instantaneously after insemination are more sensitive than the fertilized eggs may be accounted for by assuming that some group in the protein which is available for the attack of nitrogen-mustard in the unfertilized eggs becomes masked in the fertilized ones. A better and more clear understanding of the mechanism is expected following our experiments underway in connection with the tests of the difference of metabolic conditions and the enzyme systems between treated fertilized and unfertilized eggs.

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Zusammenfassung

Es wurde die Wirkung des Stickstoffsenfgases, Methyl-β-(dichloräthyl)-amino-HCl, auf befruchtete und unbefruchtete Eier der Seeigel *Arbacia lixula* und *Sphaerechinus granularis* untersucht. Die Furchungshemmung und das Stadium, bei dem die Entwicklung aufhört, hängen von der Konzentration der verwendeten Substanzen und von der Dauer der Behandlung ab. *Vor* der Besamung behandelte Eier zeigten eine ausgesprochenere Reaktion als diejenigen, die nach der Besamung behandelt wurden; auch bildeten die ersteren bei höheren Konzentrationen abnorme Befruchtungsmembranen. Die Eier, welche *nach* der Besamung behandelt wurden, zeigten ein fortschreitendes Abnehmen in der Reaktion auf die Substanz, und zwar in dem Verhältnis, in dem der Zeitabstand zwischen Besamung und Behandlung zunahm. Die Verzögerung der Entwicklung ist in Eiern, die sofort nach der Befruchtung behandelt worden waren, am größten; bei diesen ist der Prozentsatz der Eier, die eine normale Befruchtungsmembran bilden, stark reduziert; sehr häufig tritt eine Polyspermie ein.