

may be caused by sudden changes in the culture medium, involving nutritional deficiencies. After this time (from 72 to 120 h) chromosomes appear much contracted. As we have already pointed out, the abnormalities are exclusively numerical; structural changes have been found, but are not considered here. The first and fourth pairs are involved primarily when supernumeraries are present or when chromosomes are lost (84.4%), while in only 15.6% of the cells are the two major autosomes involved (Figures 2-5). This finding is worth noting in view of further biochemical studies on cells in vitro. In fact, a different gene dosage is expected for genes located in the second and in the third pair, if compared with those located in the first and the fourth pair.

We may conclude that in *Drosophila* heteroploidy commonly occurs in cultured cells, but that this condition does not follow a primary acquisition of tetraploidy. Non-disjunctions and errors of chromosome distribution are presumably the causes of the numerical abnormalities described. The present data, owing to the scope of this investigation, do not allow any comparison with those found by HORIKAWA and FOX⁶. We wish only to emphasize that these authors distinguish two types of cells (the large and the small ones). Our observations, since they refer to short-term cultures, have dealt mainly with large cells; only between 72 and 120 h may we have found also mitoses of small cells. Using our staining technique,

however, the problem of distinguishing the two cell types remains unsolved^{8,9}.

Riassunto. Cellule embrionali di *D. melanogaster*, coltivate in vitro fino a 120 h, presentano un progressivo aumento delle variazioni dei numeri cromosomici interessanti principalmente il I e il IV paio di cromosomi.

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October 4, 1965.*

⁸ One of us (S.D.) wishes to express her deepest gratitude to Prof. A. S. FOX and to Dr. M. HORIKAWA for the kind hospitality and the valuable help offered to her during her stay at the Department of Genetics, University of Wisconsin, Madison, during the fall of 1964. The authors are also grateful to Prof. A. S. FOX for critical reading of the manuscript. - The financial help of the Consiglio Nazionale delle Ricerche, Roma, is also gratefully acknowledged.

⁹ Added to the proofs. During the last months two additional evidences have been found, which substantiate the viability of the cultured cells: growth curves (till 192 h) and incorporation of tritiated thymidine (the latter finding in collaboration with the Euratom Unit for Human Radiation and Cytogenetics, Pavia, directed by Prof. M. FRACCARO).

About a Possible Mechanism Involved in the Shedding of Sea-Urchins

In spite of extensive investigations concerning the stimulus which causes the spawning of ripe sea-urchins¹⁻⁷, the problem of shedding in natural conditions is far from being understood as yet. In the experiments described, we therefore tried to elucidate the influence of spawning sea-urchins on other members of a sea-urchin population in their natural environment.

A colony of *Paracentrotus lividus* (Lam.) inhabiting rocky bottom at a depth of 2-3 m was chosen for the experiments in situ. The average density of population was estimated to be about 5 sea-urchins per m². This experimental area was in the North Adriatic near Figarola Island. Underwater observations and the removal of sea-urchins during the experiments were accomplished by free diving technique.

Dense suspensions (about 10%) of homogenized ripe male and female gonads from *Paracentrotus lividus* were prepared. The suspension was taken in a syringe of 200 ml. By diving, 5-10 ml of this suspension was delivered from the syringe close to the sea-urchins. The suspension was never delivered nearer than 10 cm to the animals. The effects of the injection of the gonad suspension were inspected by diving. The shedding which occurred in some animals could easily be registered. From the colour of extruded gametes it was easy to conclude whether the reacting animal was a male or a female. No animal was tested twice. During the whole procedure described previously the animals were neither touched nor disturbed in any other way. About 5 min after the gonad suspension was injected, the animals were lifted from the bottom without regard as to whether they reacted to the injection

with shedding or not. They were opened with a circular cut and their sex and maturity were established.

In these experiments, more than a hundred animals were tested: 51 males and 50 females. The results of these investigations are summarized in the Table.

The shedding reaction in sea-urchins (*Paracentrotus lividus* Lam.) tested with the suspension of homogenized male and female gonads, respectively

Suspension applied	Reaction	Sex and maturity of tested animals			
		Male		Female	
		Ripe	Unripe	Ripe	Unripe
Male	Shedding	-	-	24	-
	No shedding	22	2	1	4
Female	Shedding	23	-	3	-
	No shedding	4	-	14	4

¹ L. PALMER, *Physiol. Zoöl.* 10, 352 (1937).

² H. M. FOX, *Proc. Camb. phil. Soc. biol. Sci.* 1, 71 (1924).

³ H. M. FOX, *Nature* 109, 237 (1922).

⁴ M. YOSHIDA, *Annotnes zool. jap.* 25, 117 (1952).

⁵ T. MORTENSEN, III, *K. danske Vidensk. Selsk. Skr., Naturv. Math. Ser.* 9, Vol. 7, 1 (1937).

⁶ E. B. HARVEY, *The American Arbacia* (Princeton University Press 1956).

⁷ A. MONROY, cited as personal communication in HARVEY's *The American Arbacia*.

From these results it is evident that the shedding was highly sex-specific, i.e. it depended on the kind of gonad extracts used. Nearly all (23 out of 27) ripe males responded with spawning to the suspension of female gonads, and not one spawned after the suspension of male gonads was applied. On the other hand, by far the majority of ripe females (24 out of 25) spawned after treatment with the male gonad suspension, while only some of them (3 out of 17) gave a positive reaction with the suspension of female gonads also.

The results obtained are so clear-cut that no statistical analysis can improve the evidence for the specificity of the shedding reaction.

A great variety of substances cause a non-specific shedding of sea-urchins in laboratory conditions, but there is no evidence as yet for the existence of some specific biological stimuli in nature. The experiment described sug-

gests that the presence of genital products in the vicinity of ripe specimens leads to a sex-specific shedding reaction. The role of this mechanism in nature is obscure, but it can be mentioned as one of the possibilities which provides for successful fertilization.

Zusammenfassung. Experimente in situ haben gezeigt, dass durch Suspensionen der Gameten bei reifen Seeigeln (*Paracentrotus lividus* Lam.) ein Ausstossen der Gameten induziert wird. Diese Reaktion ist streng geschlechtsspezifisch.

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Der Einfluss der Adenosintriphosphorsäure auf die Strahlenpneumonitis

Sowohl nach Ganzkörper-, wie auch nach Teilkörperbestrahlungen wurde ein Absinken der Adenosintriphosphorsäure (ATP)-Konzentration in verschiedenen Organen festgestellt¹⁻⁵. LANGENDORFF et al.⁶ untersuchten daraufhin die radioprotektive Wirkung energiereicher Phosphate und phosphorylierter Vitamine. Während Adenosin, Adenosinmonophosphat, Pyridoxal-5-phosphat und Aneurinmonophosphat keine Schutzwirkung zeigten, erhöhten Synkavit, Aneurindiphosphat und Lactoflavin-5-phosphat die Strahlensensibilität. ATP zeigte dagegen bei Ratten eine deutliche Schutzwirkung. Bei subletalen Dosen erhöhte sich die Zahl der Überlebenden von 26% auf 56%. Bei Mäusen stieg die Überlebensrate von 4% auf 92,5%⁷. Um die Aufklärung dieses Phänomens bemühten sich eine ganze Reihe von Autoren⁷⁻¹¹.

Da die Schutzwirkung des ATP gegen ionisierende Strahlen bisher nur am Parameter der Mortalität eines Kollektivs geprüft worden war, untersuchten wir seine Schutzwirkung auf Einzelorgane, wobei unsere Wahl auf die Lungen fiel, wo eine genügend hohe kurative Strahlendosis oft wegen der Mitreaktion gesunder Gewebspartien nicht zu erreichen ist.

Methode und Material. Wir verwendeten weibliche Ratten eines institutseigenen Inzuchtstammes von 230–280 g Gewicht. Haltung in klimatisierten Räumen in Zweierkäfigen bei Trockenstandartkost und Wasser ad libitum. Zur Erzeugung einer Strahlenpneumonitis erhielten die Tiere ein 4,25 · 2,25 cm Feld auf den Thorax unter folgenden Bedingungen: Einfallsdosis 2000 R, Dosisleistung 200 R/min. Zur Ruhigstellung erfolgte die Bestrahlung in Urethranarkose (1,4–1,6 cm³ 20% Urethranlösung s.c.). Strahlenquelle: Gammatron I der Siemens-Reiniger-Werke mit 2000 Ci ⁶⁰Co, HWS 14,5 cm Cu, Fokus-Hautdistanz 40 cm. Da nach Angaben von KAUFMANN¹² gegen Mitte des 2. Monats post-rad. der Höhepunkt der akuten Strahlenreaktion erreicht wird, töteten wir am 45. Tag alle noch lebenden Tiere. Die Lungen wurden in Formalin fixiert. Paraffinschnitte wurden mit Hämatoxylin-Eosin und van Gieson gefärbt, zum Teil wurde auch zusätzlich die Weigertsche Fibrin- und Elastikafärbung durchgeführt.

Das Tierkollektiv wurde in 2 Gruppen unterteilt: 11 Tiere wurden nur bestrahlt und dienten als Kontrolle. 12 Tiere erhielten 12 min vor der Bestrahlung 20 mg ATP

Tabelle I. Kontrolltiere. Übersicht über die histologischen Veränderungen der Lungen am 45. Tag post-rad. mit 2000 R. Tier Nr. I, VI und IX in der Frühphase ohne Strahlenpneumonitis verstorben und daher hier nicht aufgeführt.

Nummer des Tieres	II	III	IV	V	VII	VIII	X
Perivaskuläre Entzündungen	+	+	+	+	+	+	+
Peribronchiale Entzündungen	+	+	+	+	+	–	+
Diffuse, interstitielle Entzündungen	–	+	+	–	–	+	+
Herdförmige interstitielle Entzündungen	+	–	–	+	+	–	–
Emphysem	–	+	–	+	+	+	–
Abgeschilferte Alveolar-Deckzellen	–	+	+	–	+	+	+
Ausdehnung der Entzündung	±	+	+++	+	+	+++	++

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² H. J. MAURER und K. H. PFEFFER, *Klin. Wschr.* 31, 902 (1953).

³ H. MAAS, *Strahlentherapie* 111, 79 (1960).

⁴ K. P. DUBOIS und R. F. PETERSEN, *Am. J. Physiol.* 176, 282 (1954).

⁵ V. VARGA und F. D'ONOFRIO, *Gazz. int. med. chir.* 62, 3338 (1957).

⁶ H. LANGENDORFF, U. KOCH und H. J. MELCHING, *Strahlentherapie* 99, 375 (1956).

⁷ H. LANGENDORFF und H. J. MELCHING, *Strahlentherapie* 110, 505 (1959).

⁸ H. LANGENDORFF, H. J. MELCHING und H. RÖSLER, *Strahlentherapie* 113, 603 (1960).

⁹ J. PANY, *Wiener klin. Wschr.* 72, 681 (1960).

¹⁰ N. A. HILLARP, B. HÖGBERG und B. NIELSEN, *Nature* 170, 1032 (1955).

¹¹ G. V. R. BORN, G. J. INGRAM und R. S. STACEY, *J. Physiol.* 135, 633 (1957).

¹² E. KAUFMANN, *Lehrbuch der speziellen, pathologischen Anatomie B II* (Springer Verlag, Berlin 1960), p. 1682.