About the DNA-Synthesis During the Early Development of *Paracentrotus lividus*, *Arbacia lixula* and their Hybrids

In a previous paper, BALTZER and CHEN[1] reported experiments concerned with DNA-synthesis during the early development of the sea urchins *Paracentrotus lividus* (PP), *Arbacia lixula* (AA), *Sphaerechinus granularis* (SS) and the hybrid combinations P♀ × A♂ (PA) and P♀ × S♂ (PS). The intensity of DNA synthesis was determined by measuring the amount of ^{3}H-thymidine taken up by the embryos. The figures indicate that, when comparing the same developmental stages, the rate of incorporation differs from one species to another (f.e. MyBl of PP = 120.5 cpm/100 embryos; MyBl of AA = 30.0 cpm/100 embryos). The corresponding values for the hybrid PA, in which no elimination of chromosomes occurs[2,3] are situated between those of the pure species PP and AA (MyBl of PA = 61.3 cpm/100 embryos).

This interspecific comparison, however, does not reveal the amount of ^{3}H-thymidine incorporated by the individual nuclei of these species and hybrids, because the number of nuclei and the rate of mitosis differ from species to species at any given developmental stage. The experiments reported in the present paper use an indirect approach to analyse the rate of ^{3}H-thymidine incorporation exhibited *by single replicating nuclei* of the pure species and their hybrid. These investigations are based on the figures obtained by BALTZER and CHEN[1] representing the total uptake of ^{3}H-thymidine of 100 or 200 embryos.

From the same material from which these measurements were taken, small samples were isolated for microscopical and autoradiographic examination. For each species and for any given developmental stage, it was possible to determine the average number of nuclei per embryo and to examine autoradiographically how many of these nuclei had incorporated ^{3}H-thymidine during the period of incubation. The 3 parameters total uptake of ^{3}H-thymidine per embryo (CHEN), total number of nuclei (BALTZER) and percentage of radioactive nuclei (TARDENT) permit calculation of the rate of incorporation typical for a single nucleus of a given species at a given stage of development.

Material and method. Details of cross-fertilization and rearing of the embryos were published in previous papers.

The different developmental stages of the pure species and the hybrids were exposed for 2 h at room temperature (20–22 °C) to a solution of ^{3}H-thymidine. The final concentration of the ^{3}H-thymidine solution was 4.44 μc/ml. After incubation and washing of the embryos, the DNA was extracted according to the method of SCOTT, FRACCASTORO and TAFT[4]. The radioactivity of the neutralized and dried extracts was measured in a windowless gas flow counter. In the case of PP and PA, 100 embryos provided a sufficient amount of extract, while 200 embryos of AA were needed for satisfactory readings. Samples of the same cultures were used for determining the total number of nuclei according to the method described earlier[5].

The samples isolated for autoradiographic examination, consisting of 10–20 embryos, were preserved in 80% ethanol and embedded in paraffin within a gelatine capsule. The sections (5 μ) were coated with stripping film (Kodak AR/10) and developed 4–5 days later. Staining with Haemalaun occurred after development and after the permeability of the film for the dye had been improved by treating it with alcohol 70%[6]. In analysing the autoradiograms, we established the following rules: the relative frequency of labelled nuclei was determined on those sections only which went through the centre of the embryo, thus tangential sections were not analysed. In each section we counted the total number of nuclei and the number of labelled nuclei. Nuclei were considered as being labelled when the nuclear area was covered by a group of more than 5 silver granules. The errors which such a procedure might include can be neutralized by an accordingly large number of countings. Furthermore the quantitative analysis of the autoradiographs was made by 3 different persons.

Results. The Table gives all the data concerning the 2 developmental stages of PP, AA and PA examined so far. The first group includes embryos of 16–18 h, while the

[1] F. BALTZER and P. S. CHEN, Experientia *27*, 194 (1965).
[2] F. BALTZER, Arch. Zellforsch. *5*, 497 (1910).
[3] F. BALTZER und P. S. CHEN, Revue suisse Zool. *67*, 183 (1960).
[4] J. F. SCOTT, A. P. FRACCASTORO and E. B. TAFT, J. Histochem. Cytochem. *4*, 1 (1956).
[5] F. BALTZER und P. S. CHEN, Experientia *20*, 236 (1964).
[6] A. FICQ, *The Cell* (Academic Press, New York 1959), vol. 1, p. 65.

Combination	Age h min	Stage	cpm/embryo × 10^{-2} Lit.[1]	$\bar{x}$ of the total number of nuclei/embryo	% of labelled nuclei	Average No. of labelled nuclei/embryo (abs.)	cpm/replicating nucleus × 10^{-2}
PP	18 25	MyBl/GaJ0	120.5	468.3 ± 136.4	33.5	156.88	0.768
AA	17 20 to 18 25	MyBl	30.0	240.3 ± 43.0	29.1	69.92	0.429
PA	16 30 to 18 50	MyBl	61.3	499.8 ± 86.8	40.8	203.91	0.305
PP	26 30 to 29 45	GaJ$^4/_5$	155.1	795.1 ± 195.5	29.5	234.55	0.661
AA	26 20 to 28 00	GaJ$^1/_2$	39.6	376.8 ± 63.8	27.6	103.99	0.380
PA	26 30	GaJ0–$^1/_2$	62.2	496.0 ± 134.2	27.4	135.90	0.458

MyBl, Blastula with mesenchyme cells; GaJ$^1/_2$–$^4/_5$, Gastrula, entodermal invagination completed to $^1/_2$–$^4/_5$.

second group consisted of 26–29 h old embryos. Since the development of AA proceeds more slowly than that of PP, the developmental stage of PP is somewhat advanced, especially in the group of 26–29 h. A part of the difference with respect to the total number of nuclei is due to this.

Comparing first the combinations PP and AA of the first group, we know from earlier studies[1] that embryos of PP incorporate an amount of ^{3}H-thymidine nearly 4 times as high as that recorded for AA embryos. Comparing at the same developmental stage the total number of nuclei per embryo (Table), it can be seen that PP contains nearly twice as many nuclei as AA. As indicated by the autoradiographic examination 33.5% of the nuclei of PP and 29.1% of those of AA have incorporated ^{3}H-thymidine during the incubation period. The rate of incorporation related to the single replicating nuclei is therefore – as shown in the Table – 0.768 cpm $\times 10^{-2}$ for PP nuclei and 0.429×10^{-2} for AA nuclei. This means that a replicating nucleus of PP incorporates about 79% more ^{3}H-thymidine than a replicating nucleus of AA. The hybrid PA which up to this stage shows *normal* development, gives a corresponding value of 0.305×10^{-2} cpm/nucleus, which is considerably lower than those calculated for PP and AA.

In the second group, consisting of older embryos, the results are similar at least with respect to the pure species. Again the values calculated for PP is 74% higher than those of AA (PP = 0.661×10^{-2} cpm/nucleus; AA = 0.380×10^{-2} cpm/nucleus). These absolute values differ only slightly from those recorded in younger embryos. This means that within each species the rate of ^{3}H-thymidine incorporation per nucleus remains constant all along the early development. This concordance can also be considered as being a test for the reliability of the methods used in this study.

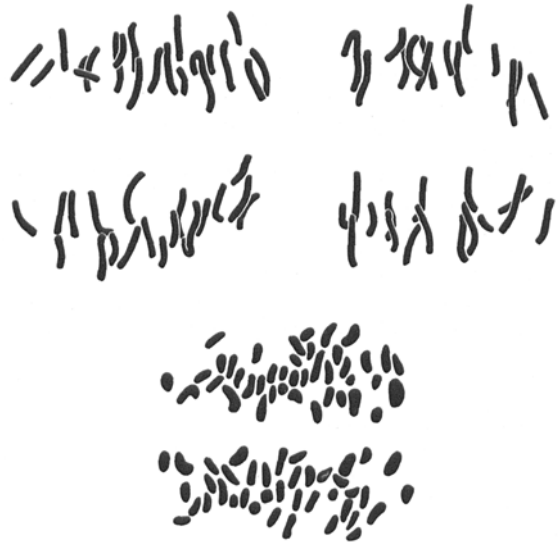
The rate of ^{3}H-thymidine incorporation of the hybrids PA of the second group entering the stage of early gastrula is now higher (0.458×10^{-2} cpm/nucleus) than that of AA and assumes an intermediate position.

Discussion. P. S. CHEN has summarized in a comprehensive article about 'nucleo-cytoplasmic interactions in morphogenesis'[7] all our previous experiments. The results reported in the present paper concerns now the DNA-synthesis of the individual nucleus. The comparison between *Paracentrotus* (PP) and *Arbacia* (AA) as to the rate of ^{3}H-thymidine incorporation during 2 subsequent developmental stages has shown that the replicating nucleus of PP incorporates 74–79% more ^{3}H-thymidine than that of AA. This means that, if ^{3}H-thymidine incorporation really is a reliable measure for DNA synthesis, the nucleus of *Paracentrotus* accordingly synthesizes more DNA than that of *Arbacia*. As we deal with related species, it is hard to believe that the genetic inventory of PP is so much richer than that of AA. The causes for these interspecific differences (comp. MIRSKY and RIS[8], ULLERICH[9]), cannot be explained on the basis of different chromosome numbers, because the diploid PP has 4 chromosomes less than AA (PP $2n = 36$, AA $2n = 40$). According to BALTZERS[2,10] observations, however, the metaphasic and anaphasic chromosomes of PP are considerably longer than those of AA (Figure). This fact, together with the demonstrated differential DNA-synthesis, could be considered as being the expression of interspecific differences of polyteny as proposed by MIRSKY and RIS[8] for other similar cases. ULLERICH[9], who found comparable interspecific differences in toads, however, rejects this hypothesis and believes that the differences in the rate of DNA synthesis is due to local duplications within individual chromosomes. The problem still remains open.

At this point it must be emphasized that our results concerning the species-specific incorporation of ^{3}H-thymidine do not agree with the findings of FICQ and BRACHET[11], who have estimated the incorporation of radioactive thymidine in AA, PP and PA by purely autoradiographic methods. They have counted the number of silver granules appearing above labelled nuclei. Using this method as a quantitative estimate for the rate of DNA synthesis, they found that the nuclei of AA incorporate twice as much ^{3}H-thymidine as those of PP. This conclusion is diametrically opposed to the results of our experiments.

A similar non-concordance concerns the hybrid PA for which FICQ and BRACHET[11] have found values which are considerably higher than those of the pure species PP and AA. Our calculations (Table), however, show that the nuclei of hybrid blastulae incorporate less ^{3}H-thymidine than those of comparable stages of AA and PP. Later in the gastrula stage, the average values for PA take an intermediate position between AA and PP. We cannot yet explain why the values for PA do not remain constant from one stage to another, but it is evident that DNA synthesis of the hybrid nuclei always remains below or within the range of that of the pure species PP and AA.

For the time being we cannot explain why our results contradict those of FICQ and BRACHET[11] to such a considerable extent. Both methods used certainly involve



Comparison of the sizes of anaphase chromosomes of first cleavage redrawn in scale from BALTZER[2,10]. (a) *Paracentrotus lividus*, chromosomes in 2 sections (cfr.[10] Table 37, Figure 1a, b). (b) *Arbacia lixula*, chromosome plates combined from 3 sections (cfr.[2] Table 29, Figure 34). × 3480.

[7] P. S. CHEN, in *Biochemistry of animal development* (ed. R. WEBER; Academic Press, New York 1967), vol. II, p. 115.
[8] A. E. MIRSKY and H. RIS, J. gen. Physiol. *34*, 451 (1951).
[9] F. H. ULLERICH, Chromosoma *18*, 316 (1966).
[10] F. BALTZER, Arch. Zellforsch. *2*, 549 (1909).
[11] A. FICQ and J. BRACHET, Expl Cell Res. *32*, 90 (1963).

possible errors. It would therefore be desirable if the problem could be tackled using yet another procedure, for example cytospectrophotometry[12].

Zusammenfassung. (1) Bei den Seeigelarten *Paracentrotus* (PP) und *Arbacia* (AA) sowie dem Hybriden PA wurde die DNS-Synthese für die Stadien der Mesenchymblastula und Gastrulation bestimmt. Als Grundlagen dienten: die an den Ganzkeimen gefundenen Werte der Inkorporation von H^3-Thymidin, die Gesamtzahl der Kerne und, an Schnitten bestimmt, der Prozentsatz der replizierenden Kernstadien. Es ergab sich, dass die DNS-Synthese *pro Kern* in den genannten Stadien (18 h und 26–29 h) bei PP rund doppelt so gross ist wie bei AA. Einzelheiten siehe Tabelle. (2) Es werden DNS-Synthese und Chromosomengrösse der beiden Arten verglichen und wird auf den Gegensatz zu den Befunden von FICQ und BRACHET[11] hingewiesen.

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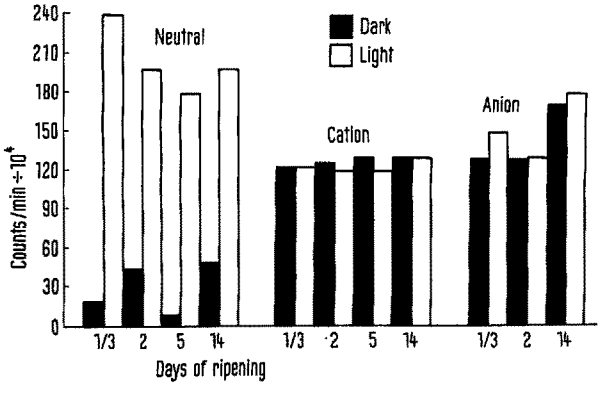
Ripening Tomatoes: $C^{14}O_2$ Uptake by Green Tomato Fruit

The tomato fruit receives sugars and phosphates from the leaves of the plant (MCCOLLUM[1]). Data reported by DALAL[2] showed that the tomato fruit contains sufficient chlorophyll to synthesize much of its carbohydrate supply. Studies conducted by these investigators showed that when tomatoes were treated with light, the CO_2 given off by the green fruit was less than from dark treated fruit.

To test the hypothesis that the tomato fruit were using CO_2 a set of samples was prepared and treated with $C^{14}O_2$. Uniformly mature green tomato fruits were placed in a quart size ripening chamber fitted with surgical tubing to facilitate the injection of the radioactive gas with a hypodermic needle. The $C^{14}O_2$ was generated from barium carbonate by reaction with lactic acid in a mercury filled U-tube. Two ml of the $C^{14}O_2$ was injected into each of the sealed ripening chambers. Twelve of these chambers were prepared. Half of them were covered with aluminum foil for the dark treatment and the other half placed under a light source of 100 foot candles. After 8 h of exposure the ripening chambers were attached to respirometers and air passed over the fruit. Samples were preserved in ethyl alcohol after 8 h, 2 days, 5 days, 10 days, and 14 days of ripening. The samples were separated on ion exchange columns into a neutral, anion, and cation fractions. The radioactivity of the fractions was determined on a liquid scintillation counter.



Radioactivity of the neutral, cation and anion fractions of tomato fruit treated with $C^{14}O_2$ for $^1/_3$ day prior to ripening.

The Figure shows the counts/min of radiation given off by the 3 fractions separated from tomato fruit treated 8 h with $C^{14}O_2$ in light and dark. All 3 fractions contained considerable radioactive label. However, only the neutral fraction showed significant differences between treatments. In this case those fruit treated in the light showed a much higher label.

The uptake of carbon-14 dioxide into the cation and anion fractions of the fruit was equal in light and dark. The amount of label in the cation fraction essentially remained constant, whereas, the carbon 14 label in the anion fraction increased somewhat.

This information lends itself to speculative interpretation on the basis of the mechanisms of photosynthesis, metabolism, and fruit ripening. First it makes the fruit much more of an independent unit, and the possibility that a detached tomato fruit could be kept alive and growing seems apparent. The green tomato fruit, at least in the first 8 h of ripening, is capable of photosynthesis.

Further, this study shows that the tomato is actively incorporating carbon dioxide into its components by dark fixation. The active synthesis of amino acids occurs after the tomato is detached from the plant.

The results of this experiment also show that information received from respiration studies that measure either carbon dioxide evolved or oxygen uptake of green tomato fruit could be misleading depending on the experimental conditions.

Zusammenfassung. Die neutrale Fraktion grüner Tomatenfrüchte zeigt nach Fütterung mit $C^{14}O_2$ im Licht eine höhere Radioaktivität als nach der Fütterung im Dunkeln. Die grüne Tomatenfrucht ist zur Photosynthese befähigt.

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Utah State University, Logan (Utah USA), 20th March 1967.

[1] J. P. MCCOLLUM, Proc. Am. Soc. hort. Sci *75*, 611 (1960).
[2] K. B. DALAL, D. K. SALUNKHE, A. A. BOE and L. E. OLSON, Fd Res. *30*, 504 (1965).