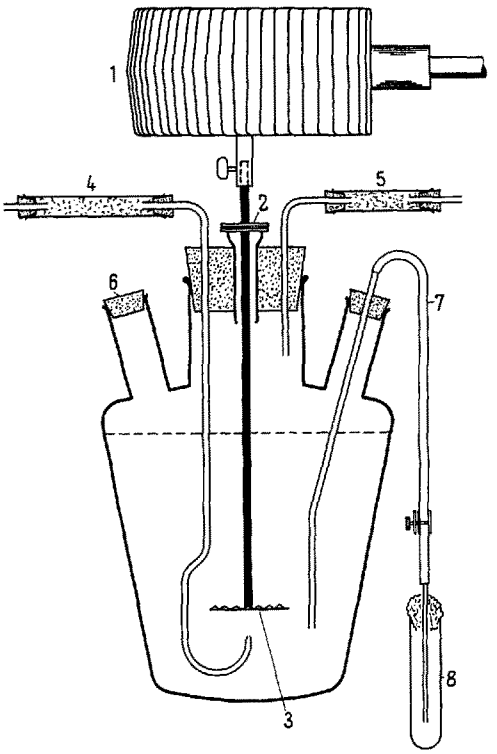
Another distinctive advantage of the apparatus is that air is divided up in very small bubbles if the air inlet is centred underneath the stirrer plate; thus a highly efficient transfer of oxygen is ensured. All other fittings in the culture vessel can be seen in the Figure. For most experiments a 6-l flask with 5-l working capacity and stirrer plates of glass or metal up to 65 mm diameter have been used. Larger volumes of culture (up to 10 l) can be satisfactorily stirred with the smallest vibrator supplied. Larger models are made by the manufacturer.



Assembly of the Vibro Mix Apparatus. (1) Vibrator, (2) sealing gland, (3) stirrer disc, (4) filter for air coming in, (5) filter for used air let out, (6) inoculation orifice, (7) siphon for sampling, (8) sterile tube to prevent contamination of the siphon.

For most purposes it is satisfactory to estimate the amplitude by eye and adjust accordingly. If the growth and fermentation characteristics depend very strongly upon the mechanical stress imposed, stroboscope and telescope can be used as aids for an accurate adjustment of the amplitude. There are generally no variations of the amplitude over a period of about two days.

A suitable criterion for a method of cultivation is the behaviour of the culture itself. With the described apparatus exponential growth (i.e. unrestricted growth with constant rate of multiplication) can be obtained over a wide range of the growth curve. In substrate A or A_4[3,4] the average rate of multiplication of *Aspergillus oryzae* was 0.28 to 0.3 duplications of dry weight mycelium/h, this rate remaining constant up to about 160 mg dry weight mycelium/100 ml. At 37°C the rate of multiplication is considerably higher although the exact figure has not been estimated. The results compare favourably with those obtained by other authors: 0.109 to 0.2 duplications/h for *Penicillium chrysogenum*[5]; 0.264 duplications/h for *Aspergillus ochraceus*[6].

Zusammenfassung. Ein Apparat zum Mischen und Umwälzen von Flüssigkeiten durch Vibrationen (Vibro-Mischer), besonders zur submersen Kultivierung von Mikroorganismen wurde entwickelt. Eine sehr leistungsfähige Belüftung konnte ohne poröses Material zur Feinverteilung der Luft erzielt werden. Mit dieser Methode wurde bei *Aspergillus oryzae* eine exponentielle Vermehrungsphase mit etwa 0,3 Gewichtsverdoppelungen pro h bei 25°C über einen weiten Bereich der Wachstumskurve festgestellt.

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Department of Applied Microbiology and Biology, The Royal College of Science and Technology, Glasgow (Scotland), December 19, 1963.

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STUDIORUM PROGRESSUS

Further Morphological and Biochemical Studies on Normal and Hybrid Embryos of Sea Urchins[1]

The present authors have studied in previous experiments the synthesis of desoxyribonucleic acid (DNA) during normal and hybrid development of the sea urchin embryos[2–6]. In addition, the metabolic activity of ribonucleic acid (RNA) has been analysed by using the technique of isotopic labelling[7]. The present paper deals with further results of our RNA experiments which have been extended to other hybrid combinations. At the same time it appeared very desirable to have detailed data about the numbers of nuclei in different developmental stages of both the pure species and the hybrids. In the following, we shall summarize the results of the nuclear countings in the first section; the biochemical findings will be presented in the second section.

I. Counting of Nuclei (BALTZER)

Technique. For counting the nuclei squashed preparations were used. The embryos were fixed for $^1/_2$ to $1^1/_2$ h in picro-acetic acid (Boveri), washed several times in distilled water and stained for $^1/_4$ h in Mayer's Haemalum. Then they were dehydrated in alcohol and squashed under a small cover-glass in a minimum of xylol and Canada balsam. By squashing, the nuclei were distributed in a

single layer and the counting was relatively but not always easy. The following developmental stages were used (culturing temperature in all cases 18–18.5°C): (a) Swimming blastula (Bl) at the age of 15–21 h. (b) Gastrula with half invaginated gut (Ga J $^1/_2$). This stage is in PP 21–21 $^1/_2$ h old[8]. (c) Prism (Pri) to young pluteus, Pl-1, with first developing anal arms, PP: 37–41 h. (d) Old pluteus (Pl-2), PP: 47–48 h.

It is evident that our study was started at a relatively late blastula stage. This was due to the fact that only by beginning from this stage could the swimming hybrids be cleanly separated from the sediments on the bottom of the culturing dishes, which contained a large amount of unfertilized eggs, abnormally developed cleavage stages and also clumps of sperms.

The nuclear countings are summarized in the Table. As can be seen, the three pure species studied have different rates of development and do not reach the corresponding stages at the same time. At 48 h the embryos of PP are at the late pluteus stage (Pl-2) with long anal arms, whereas those of AA have reached only the prism or the young pluteus stage. At this time, the SS embryos are still gastrulae (Ga J $^1/_1$, with fully invaginated gut).

Comments to the Table. (1) Nuclear number in the pure species PP, AA, SS: From the swimming blastula to the pluteus stage, the numbers of nuclei increase four times in PP, but there is only a two-fold increase in AA and also in SS. To explain such a discrepancy, different factors have to be considered: compared to PP the increase of nuclear number in AA is in itself lower (as can be seen by the mitotic number). In SS the pluteus stage is reached only at a markedly later period, and consequently the formation of the ciliary bands in the anal arms and the oral lobes, which become rich in nuclei, is also delayed.

(2) Nuclear number in the hybrids PA and AP: *PA*. As shown by the Table, and as already described for PA by WHITELEY and BALTZER[2], the development of both reciprocal combinations stops at the late blastula stage or at the beginning of gastrulation. The embryos then live for 1–3 more days (maintenance period) without or almost without further development. The time and the grade of inhibition vary quite considerably. Only an extremely small percentage of embryos in individual cultures reach a delayed pluteus stage. These 'break-throughs' need not be considered here.

[1] Our work was supported by a grant from the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung'. We express our sincere thanks to the 'Nationalfonds' and also to the authorities at the Zoological Station at Naples for generous help and supply of materials and facilities. – This paper is thankfully dedicated by F. BALTZER to F. E. LEHMANN for his kind hospitality in the Bernese Institute.

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[8] Abbreviations: PP = *Paracentrotus lividus*, AA = *Arbacia lixula*, SS = *Sphaerechinus granularis;* PA = P♀ × A♂, AP = A♀ × P♂, PS = P♀ × S♂, h = hatching. BlMy 1–2 = early and late blastulae in which the primary mesenchyme cells begin to move in until the formation of a ring. The development of the gut is given in numerical ratios: GaJ 0 = gastrula with beginning of gut invagination, GaJ $^1/_4$... = gastrula with $^1/_4$ invaginated gut etc. Pri = prism. Pl-1 = pluteus with short, Pl-2 = pluteus with longer anal arms. For illustrations of these stages, compare WHITELEY and BALTZER[2].

Average numbers of nuclei at different developmental stages of normal and hybrid sea urchin embryos

Developmental stage[a]	Age in h after fertilization[a] (18°C)	No. of countings	Average number of nuclei	Developmental stage	Age in h after fertilization (18°C)	No. of countings	Average number of nuclei
PP 1962, 1963				AA 1962, 1963			
Bl with immigrating My	15 h 14	4	391	Bl with immigrating My	16 h 40	2	318
GaJ 1/2–J 4/5	22 h 00–26 h 30	5	700	GaJ 1/3–J 2/3	24 h 00–26 h 40	4	390
Pri–Pl-1	37 h 55	4	761	Gaj 2/3–Pri	38 h–40 h	2	605
Pl-1	41 h 00	5	956	Pri–Pl-1	47 h 35	3	685
Pl-2	48 h 00	2	1429	Pl 1-2	66 h–72 h	2	1050
				Pl-2	77 h 55	2	1473
PA 1962, 1963				AP 1963			
Bl with immigrating My	16 h 30–18 h 50	7	492	Bl with immigrating My	21 h 30	2	307
GaJ 1/4–J 1/2	24–26 h 40	6	533	GaJO–J 2/3	26 h 00–32 h 30	5	357
GaJ 1/2–J 4/5	48 h 00	2	641	GaJ 1/2–J 2/3	38 h 20	2	304
GaJ 1/3–J 4/5	61 h–65 h	2	982	GaJO–J 1/3	45 h 00	3	402
SS 1963 and earlier				PS 1963			
Bl, thick wall, before My moving-in	20 h 40	1	414	Bl with elim. chrom. fragments in blastocoel	20 h 35	1	339
Bl with immigrating My	24 h 20	2	431	Bl with deg. mat. and My. GaJO	25 h 05–25 h 25	3	411
GaJ 1/3	27 h 00	–	–				
Ga with gut fully invag.	41 h 00	1	669				
	47 h 15	2	692	As above	41 h 05	2	517
					48 h 45	3	670

[a] The hours in the second column indicate the time of fixation for countings and RNA-determinations. They do not show precisely the limits of the developmental stages given in the first column. GaJO: first beginning of Gastrulation.

With the inhibition of development, the nuclear number of PA also remains behind that of the maternal controls. Nevertheless, there occurs a certain increase in the number of nuclei in this hybrid: at $16^1/_2$–19 h 492, at 48 h 6411, and at 65 h 982 nuclei were counted.

AP. On the average the developmental potency of this hybrid is rather low. This agrees with the fact that AA, too, develops worse than PP (BERNHARD[9]). Its nuclear numbers are the lowest of all hybrids considered. The swimming blastula aged $21^1/_2$ h has 307 nuclei (AA about 350), and the latest stage at 45 h thus far counted has 402 nuclei (AA 685, PP 1429).

The results of the nuclear countings have to be compared with those of the DNA synthesis given in Figure 3 in the previous paper by BALTZER, CHEN, and TARDENT[6]. As a whole the increase or the restriction in the nuclear number parallels that in the content of DNA: at the first developmental stages the DNA, as well as the nuclear values, are still close to those of the maternal species, and then both of them remain behind the values of the maternal controls.

(3) Nuclear number in the hybrid PS: The reciprocal hybrids PS and SP behave entirely differently. SP develops with the diploid chromosome set without difficulty to the pluteus stage with an intermediate skeleton. This combination is not included in the present study. In the reciprocal cross PS about 90% of the paternal chromosomes are eliminated at the first cleavage mitoses (BALTZER[10], BALTZER, and CHEN[4]), and, owing to this elimination, its development becomes abnormal at a very early stage. In embryos aged 8 h, i.e. distinctly earlier than the appearance of anomaly in the P/A-hybrids, the eliminated chromatin materials together with some cytoplasm are extruded in the form of vesicles or fragments into the blastocoel. As a result, the blastula contains from the beginning degenerated material. However, the embryo, as far as can be observed, still has a normal moving-in of the primary mesenchyme cells, although in most cases there is no formation of the typical mesenchyme ring. As the P/A hybrids, the PS embryos become blocked at the gastrula stage and also have a maintenance period of about 2 days. In rare cases plutei are formed too. This abnormal type of development is again bound to a reduction in the replication of the nuclei. Whereas the nuclear numbers in SS from the swimming blastula at 20 h of age to the fully invaginated gastrula (GaJ $^1/_1$, 47 h 15) increase from 414 to 692, the corresponding stage of PS with eliminated chromosomes begins already with a smaller number of nuclei (PS at $20^1/_2$ h with 339, to be compared to PP with about 500 nuclei). Later on the hybrids remain as inhibited My-blastulae or -gastrulae at 48 h with 670 nuclei far behind PP.

The DNA data (see Figure 6 in BALTZER and CHEN[4]) correspond to the results of the nuclear countings.

II. Metabolic activity (isotopic labelling) of RNA (CHEN)

As mentioned above in connection with our studies on the biochemical properties of sea urchin hybrids, we have analysed in detail the metabolic changes of nucleic acids during both normal and hybrid development[2–6]. The rate of DNA synthesis is most rapid in PP, slower in SS, and much slower in AA. Similar results have been reported by previous investigators for many other sea urchin species, although the magnitude of relative increase varies (see references in WHITELEY and BALTZER[2], and CHEN[11]).

Concerning the hybrids our hitherto results can be summarized as follows: the DNA synthesis in the cross PA proceeds normally as that in the PP controls until the late blastula or the early gastrula stage. At the beginning of morphogenetic inhibition, i.e. during gastrulation, it becomes distinctly reduced, and at the maintenance period the rate values are about intermediate between both parental species. The DNA content of the reciprocal cross AP is exactly the same as that of the maternal controls until about 25 h after fertilization, and at the period of arrested development it remains at a level of only about 50% of AA. For the combination PS 16–17 of the 20 paternal chromosomes are eliminated at the first cleavage[6]. Accordingly, the development of PS changes very early to a nearly haploid type. There is an inhibition of DNA synthesis as early as 5 h after fertilization. Its content amounts to only about 50% of PP in the arrested stereo-blastulae and stereo-gastrulae. The reciprocal cross SP is viable and its DNA content appears also normal. These results demonstrate clearly that the pattern of DNA synthesis is different according to the parental species used, and corresponds closely to the morphogenetic capacity in PA and AP and the cytological behaviour in PS.

As already shown in section I, the increase in the DNA content generally parallels the increase in the nuclear number during both normal and hybrid development.

In contrast to DNA, according to our previous data the total content of RNA in all three species remains largely constant up to the pluteus stage (see Figure 7 in BALTZER and CHEN[4] and Figure 4 in CHEN, BALTZER, and ZELLER[5]). The same is true for the hybrids which keep the constant normal values of the maternal species, even though their DNA contents are reduced to various degrees. This latter result seems to be at variance with the current concept that DNA serves as the template for the formation of RNA which in turn controls the synthesis of proteins. In an attempt to clear this point experiments have been carried out by us to follow the rate of incorporation of labelled adenine into RNA during the development of both normal and hybrid embryos.

It is known that intact living sea urchin embryos, as well as cell-free systems prepared from them, are able to assimilate labelled metabolites from the surrounding medium[12–14]. The uptake process is apparently most active at the mesenchyme blastula stage[15]. According to HULTIN[16], there are qualitative changes in sea urchin ribosomes even soon after fertilization, and these activated ribosomes greatly enhance the *in vitro* protein synthesis. Furthermore, information is now available indicating that new synthesis of messenger RNA in sea urchins takes place before gastrulation[17–20]. These facts suggest that there are considerable changes in the metabolic activity of RNA, even though its total quantity remains fairly constant. Our own results, which have been briefly dealt with in a previous paper[7], are in agreement with this conclusion.

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In the following we shall report the results of some further experiments, which demonstrate that the RNA turnover in the hybrids is distinctly reduced compared to that in normal embryos. In addition to the three parental species PP, AA, SS and the hybrid PA, the present work includes also the combinations AP and PS. Hybridization and counting of the samples were carried out by BALTZER. 100 embryos were used in each analysis for PP and SS and the hybrids PA and PS, and 200 embryos for AA and the hybrid AP. The labelled precursor was adenine-8-C^{14}-sulfate which has a radioactivity of 28.3 mC/m*M* and was obtained from the Radiochemical Centre, Amersham. Each sample with a counted number of embryos was incubated for exactly 40 min at room temperature (20–23°C) in an incubation medium which contained the labelled adenine and had a final concentration of 4.17 μC/ml. For extracting RNA we used a modified microchemical method originally described by SCOTT, FRACCASTORO, and TAFT[21]. The radioactivity of the RNA extract was measured by a windowless gas-flow counter (Tracerlab). For details of the analytical procedures, the previous paper by CHEN and BALTZER[7] should be consulted.

It should be mentioned that according to our experience, and under the present experimental conditions, the incorporation of labelled adenine into DNA is negligible. Working under similar incubation conditions and using the autoradiographic technique, MARKMAN[15] also reported that labelled adenine was mainly incorporated into RNA. After the treatment of the labelled embryos with RNAse, he found that the radiation was greatly reduced, although there were large variations.

Another important point for experiments of the present type is the possibility of bacterial contamination of the incubation medium. As pointed out by NEMER[22], this is negligible compared with the incorporation ability of the living embryo. This has also been confirmed by our own control experiments.

The overall data of adenine incorporation during the development of the parental species PP and AA as well as the two related reciprocal hybrids PA and AP are shown in Figure 1. Since the results of the present study are essentially the same for PA as those obtained in 1962, values reported in our previous paper are also included. For PP, in agreement with MARKMAN[15,23], we observed a rapid uptake of the isotope from the mesenchyme blastula to the beginning of gastrulation (earlier stages not studied). Thereafter there are at first some variations, and it then increases again, though more slowly, until the pluteus stage. Compared to PP, the absolute rate of incorporation is very low in AA. Furthermore, it shows only a slight increase up to the time of gastrulation and remains at about the same level until as late as 55 h after fertilization. This illustrates that the metabolic activity of RNA is very similar to the patterns of DNA synthesis in these two sea urchin species, as reported earlier.

In regard to hybrids, the situation appears entirely different. For PA at about 21 h after fertilization, i.e. at the beginning of gastrulation, its rate of adenine incorporation is still close to that of the PP controls. During the rest developmental period, there is a continuous decrease of the incorporation rate, but even at 50 h of age the values are still higher than those of the corresponding paternal controls (AA). For the reciprocal cross AP the rates of adenine uptake are consistently lower than those of both parental species at all stages thus far examined by us. Even before gastrulation the incorporation is definitely reduced compared to the AA controls, although during this period its DNA content has been shown to be still normal. However, the overall picture indicates that the metabolic activity of RNA in this hybrid is inhibited in all stages to a lesser extent than the DNA synthesis: the relative incorporation in AP amounts to 75% of AA, whereas the DNA synthesis is only about 50% (compare the lowest curve in Figure 1 in the present study and that in Figure 3 in BALTZER, CHEN, and TARDENT[6]).

The radiation data for the hybrid PS and parallel measurements for the two parental species are summarized in Figure 2. The incorporation rate in SS is similar to that

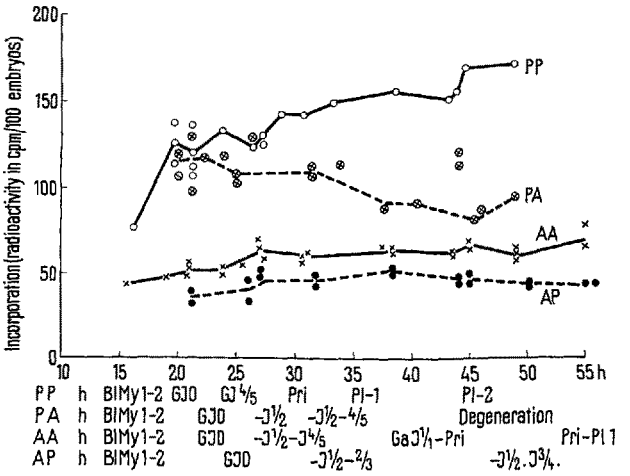

Fig. 1. Incorporation of ^{14}C-adenine into RNA during the development of PP (○), AA(×) and the hybrids PA (⊗) and AP (●). In addition to data reported in our previous paper[7], the curves include two new series of each hybrid combination (63.5, 63.12 for PA and 63.7, 63.14 for AP) and the parallel parental controls. For abbreviations of developmental stages see [8].

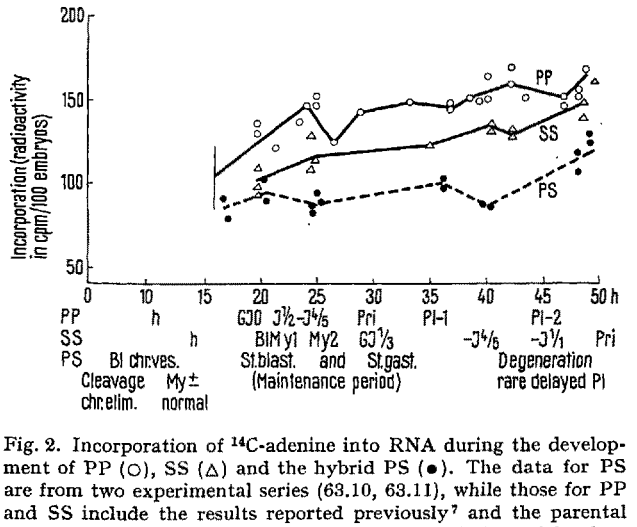

Fig. 2. Incorporation of ^{14}C-adenine into RNA during the development of PP (○), SS (△) and the hybrid PS (●). The data for PS are from two experimental series (63.10, 63.11), while those for PP and SS include the results reported previously[7] and the parental controls of the two new series. For general abbreviations of developmental stages see [8]. Bl. chr. ves. = Blastula with chromatin vesicles in wall or blastocoel. chr. elim. = elimination of chromosomes.

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in PP, but obviously lower at all stages. This is because the development of this species proceeds more slowly (see the Table of BALTZER in section I). There are considerable variations in the incorporation rate of the hybrid PS. Nevertheless, the data available indicate clearly that its RNA turnover is greatly reduced compared to both parental species. As with DNA content, this is detectable even in the blastula stage (see Figure 3 in CHEN, BALTZER, and ZELLER[5]). Similar to AP the inhibition of RNA activity is again less pronounced than that of DNA synthesis (68% instead of 50% of PP).

From the results described above the following conclusions can be drawn: (1) During normal development there is an increase in the metabolic activity of RNA, even though its total quantity remains fairly constant. In all three sea urchin species there is a striking similarity in the pattern of RNA turnover and that of DNA synthesis. (2) For the hybrid combinations there is a definite reduction in the metabolic activity of RNA in contrast to its total content which has been shown to be as normal as the maternal controls. In PA it decreases gradually at the maintenance period, and in AP and PS it is lower than that in both parental species at all stages. It should be mentioned that DNA synthesis in these hybrids is also inhibited to various degrees. Therefore, in both cases, normal as well as hybrid development, there is a close relation between RNA turnover and DNA synthesis.

Results of various investigations into RNA turnover in growing cells have demonstrated that there is probably little or no degradation and resynthesis of cellular RNA (for references see BERG[24]). On the other hand, as already mentioned, recent work suggests that new synthesis of messenger RNA occurs in the sea urchin blastulae[17–19], or still eavlier[20]. Since the messenger RNA is unstable and occurs only in small quantities compared to the bulk of ribosomal RNA (see MIRSKY[25]), it would be hardly detectable by the straight chemical determination of total RNA. The sensitive isotopic technique appears more adequate to disclose such changes, as has been shown in the present study. Unfortunately, we have no information as to what extent the synthesis of messenger RNA or other macromolecular compounds was involved in the incorporation process studied by us. Our data also do not allow us to decide whether the labelled adenine was incorporated intonuclear or cytoplasmic RNA. According to MARKMAN[15], this precursor is mainly incorporated into the nuclei, but it is likewise possible that there are exchange reactions of RNA in the cytoplasm[26–28], especially at the mesenchyme blastula and gastrula stages. The latter possibility has to be considered in view of the results obtained for the hybrid combinations AP and PS, whose relative rate of isotope incorporation, as already mentioned, is higher than their DNA synthesis.

Zusammenfassung. (1) Es wurde bei den Seeigelarten *Paracentrotus* ♀♂, *Arbacia* ♀♂ und *Sphaerechinus* ♀♂ sowie den drei Bastarden *Par* ♀× *Arb* ♂, *Arb* ♀× *Par* ♂ und *Par* ♀× *Sphaer* ♂ für eine Reihe von Entwicklungsstadien die Anzahl der Kerne pro Keim bestimmt (Tabelle). Der morphogenetischen Hemmung der Bastarde entsprechen herabgesetzte Kernzahlen und parallel eine Herabsetzung der DNS-Synthese. (2) Es wurde bei den genannten reinen Arten und den Bastarden der *Umsatz der RNS* bestimmt. Er geht in allen Fällen, bei den reinen Arten wie bei den Bastarden, der DNS- Synthese parallel.

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CONGRESSUS

Czechoslovakia

G. J. MENDEL Memorial Symposium 1865–1965

Brno/Brünn, August 1965

At the beginning of August 1965, a G. J. MENDEL Memorial Symposium will be held in Brno/Brünn, Czechoslovakia, under the patronage of the Czechoslovakian Academy of Sciences. Two days will be reserved for scientific lectures, and two days for visiting the Mendel Institutes.

A Symposium on Mutation questions will be held in Prague immediately after the Mendel Memorial Celebrations.

Committee of Organization: Professor Dr. B. NĚMEC. Secretariate: Dr. M. SOSNA, Na cvičišti 2, Prague (Czechoslovakia).

Switzerland

Invitation to the Symposium on Experimental Gerontology

Basel (Switzerland), from Friday, October 23rd to Sunday, October 25th, 1964

European Biological Section of the International Association of Gerontology

Preliminary Programme: Metabolism, Genetics, Connective Tissue and Muscle, Experimental Psychology, Central Nervous System, and Sensory Organs.

Registrations and titles of papers with abstracts of about one typed page, double spaced, should be sent by September 1st, 1964 to the Secretary of the Symposium, Institute of Experimental Gerontology, Nonnenweg 7, Basel (Switzerland).

Prof. F. BOURLIÈRE, Paris

Prof. F. VERZÁR, Basel