of gene amplification[7]. There is a clear indication that L-triiodothyronine, though responsible for the amplification of ribosomal genes in cultured human liver cells, renders no increased net synthesis of ribosomal RNA[16].

From the present results, it can further be seen that the amount of ribosomal DNA in later developmental stages of liver remains constant. This is consistent with other data concerning gene dose in several types of tissue of adult rat varying in r-RNA synthesis[17]

From all the data, it can be concluded that amplification of ribosomal genes seems not to be a physiologically regulatory mechanism operative in somatic cells. This also seems to be true of the amplification of structural genes, because it was found that the number of globin genes in hemoglobin synthesizing cells was the same as in other differentiated cells like liver[18]. Moreover, no difference in the number of genes coding for the constant part of immunoglobulins in homologous liver or myeloma DNA could be observed[19].

Summary. The content of ribosomal DNA in mice liver at the beginning as well as near the end of the hematopoietic period was measured by RNA/DNA-hybridization in solution. At both stages the amount of ribosomal DNA was the same and comparable to that of postnatal liver.

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Oocyte Maturation in vitro: Contribution of the Oviduct to Total Maturation in *Xenopus laevis*

The process which transforms an amphibian oocyte into a fertilizable egg may be subdivided into several stages. The first visible external evidence that maturation has started is the presence of the maturation spot. In *Rana pipiens*, this takes 13–18 h after contact with progesterone, 36–40 h to reach the metaphase of the second meiotic division[1], and up to 68 h to attain cleavage capacity[2].

Xenopus laevis oocytes, progesterone-matured in vitro, require about 24 h after contact with progesterone to reach cleavage capacity[3]. Yet, *X. laevis* females frequently are able to shed fertilizable eggs as soon as 7 h after injection of gonadotropic hormones. The present investigation calls special attention to the contribution of the oviduct during maturation. The criterion to judge total maturation was fertilizability of the egg and normal development.

Material and methods. Part of the ovary of *Xenopus laevis* females (which contained oocytes without any pigmentation on their vegetative pole) was dissected as described by SCHORDERET-SLATKINE[4]. The oocytes were defolliculated with watchmaker's forceps in De Boer's solution[5] stored in the same solution supplemented with 10% fetal calf serum. Progesterone was added as described by MERRIAM[6]. After 4 h most of the oocytes had started maturation as judged by the presence of the maturation spot. For the experiments summarized in Table I, the oocytes were divided into 2 equal lots: one was allowed to stay for 3 h in the maturation medium described above, the other lot was transferred into the body cavity of a foster female[7] immobilized with MSS 222. *Xenopus laevis petersi* females were used as foster females because they shed eggs heavily pigmented on the vegetative pole. The foster females were injected with 450 Gonadotrophin (Organon, Holland) 10 h before use. They were squeezed periodically during 3 h after having been injected with donor oocytes. The latter appeared, interspersed among regular eggs of the host, during this period. The oocytes were then exposed to tap water which provokes in fully matured (unfertilized or fertilized)

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Table I. Oocytes matured with progesterone (criterion: presence of the maturation spot): Comparison between oocytes which were and were not in contact with an oviduct of a foster female

	Experiment No.				Totals		Cortex contraction (%)	Abortive cleavage (%)
	1	2	3	4				
Oocytes transferred	79	34	150	268	531			
Oocytes shed	45	9	92	150	296	(100%)	49.8	53.3
Controls (oocytes incubated in medium a or b)	79[a]	34[a]	160[a]	263[b]	536	(100%)	0.6[c]	—

For each experiment the oocytes transferred to the foster female, and the oocytes incubated in medium a or b, were taken from the same donor female. [a] De Boer's solution + progesterone + fetal calf serum. [b] Serum prepared from 3 ovulating females. [c] The oocytes showed cortex contraction only at the time where the shed oocytes showed abortive cleavage (80–110 min instead of 5–15 min after contact with tap water). The controls (no contact with the oviduct) reach the stage attained within 3 h by shed, in vitro matured oocytes only after 20–24 h (see also ref. [3]).

Table II. Developmental capacity of oocytes (matured in vitro with progesterone) after shedding by foster females and 'artificial' fertilization

	Totals	%
Oocytes shed	296	100
Activation reaction	158	53.3
Blastula stage	30	10.8
Hatching tadpoles	20	6.7
Feeding tadpoles	13	4.4
Metamorphosed frogs	8	2.7

eggs, spontaneous activation, i.e. cortex contraction and cleavage (in unfertilized eggs cleavage is abortive). The two types of *Xenopus laevis laevis* oocytes, those which had passed through an oviduct within 3 h and those which had been kept in maturation medium for 3 h, were so treated and their behaviour compared. Oocytes which had passed the oviduct were fertilized following WOLF et al.[5]. In addition to the parallel storage of oocytes in maturation medium (a), a second control was done as follows: 3 ovulating females were sacrificed, and their serum prepared. Oocytes matured with progesterone were transferred into this serum (medium b) after the maturation spot had appeared, and incubated for 3 h. All experiments were realized at 21–22 °C (temperature of the solutions used).

Results. Tables I and II.

Discussion. The process of maturation not only induces completion of meiosis and the transformation from the cytoplasmic state of an oocyte (RNA synthesis) to the cytoplasmic program of an egg (DNA synthesis), but also acts on the oocytes membrane (for review see[8]). The present results suggest that cortex maturation in vivo depends on contact of the oocyte with the oviduct, since the controls did not contain fully mature eggs. This observation is corroborated by the possibility of raising the low metabolic activity of a body cavity egg to the high level of a shed egg by treating the body cavity eggs with extracts from the uppermost part of the oviduct[9].

Summary. Contact of progesterone matured oocytes of *Xenopus laevis* with the oviduct reduces the time necessary to attain cleavage capacity from 24 h to 3 h. Full maturity has been demonstrated by normal development of the matured eggs after fertilization.

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Nuclear Envelope Changes Related to Cell Activation in *Helianthus tuberosus* L.

Nuclear pore complexes are sites of communication between nucleus and cytoplasm through which macromolecular exchange is effected[1]. Although the structural organization of the pore complex appears to be similar in most cell types, there is considerable variation in the number of pores in the nuclei of different species and tissues[1]. Furthermore pore frequency and pore number per nucleus can increase in a given cell type with an increase in its metabolic activity[2–5].

The activation of plant storage tissues is accompanied by many changes[6] including a rise in RNA synthesis, an increase in nucleolar size, polysome formation and protein synthesis. A thin-section study of *Daucus carota* root cells also revealed a doubling of nuclear pore frequency[7], suggesting that nuclear pore number might be an important controlling factor in the process of cell activation. In this preliminary report, we describe the results of an investigation into nuclear envelope ultrastructure of dormant and activated *Helianthus tuberosus* tuber cells, in which the freeze-fracture technique was employed to permit a more accurate analysis of nuclear pore frequencies.

Materials and method. Explants of *H. tuberosus* tuber tissue were prepared and incubated, as described previously[6]. Nuclei were extracted from dormant tissue, and tissue incubated for 24 h (termed activated tissue in this report), fixed in 0.1 *M* cacodylate buffered 2.5% glutaraldehyde, glycerinated, frozen in melting freon 12, and freeze-fractured using the BULLIVANT-AMES method[8]. Cleaned replicas were examined with an AEI EM6B

Frequency of nuclear pores in nuclei from *Helianthus tuberosus* tuber cells

	Nuclear pores ($\mu m^{-2} \pm SD$)	Sample size
Dormant cells (0 h incubation)	11.3 ± 1.8	363 μm^2 of nuclear envelope from 24 nuclei
Activated cells (24 h incubation)	11.9 ± 1.9	191 μm^2 of nuclear envelope from 34 nuclei [a]

[a] The smaller total area of nuclei sampled in activated cells results from the reduced frequency of large areas of face fractures of the convoluted nuclear envelopes.

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