Unchanged r-RNA-Gene Dose in Mice Liver Cells of Different Developmental Stages

A marked stimulation of RNA-synthesis is noticed either in response to hormones[1,2] or during certain developmental stages in somatic tissues[3]. During oogenesis, in many animals, the period of increased r-RNA-synthesis is known to be preceded by the amplification of r-RNA genes[4]. However, with regard to somatic tissues in eukaryotic organisms, the occurence of amplification of genes, coding for ribosomal RNA, still remains controversial. Such an amplification was shown to be present in the macronucleus of *Tetrahymena pyriformis*[5], as it has also been reported to result in cultured human liver cells after exposure to L-triiodothyronine[6] or in the regenerating lens of *Triturus*[7]. In thyroid hormone-treated liver cells, this event was found to be accompanied by an increase in the number of nucleoli in the nucleus[6]. A similar increase in the average number of nucleoli in the liver of thyroidectomized rats, however, was not linked with an increase in the number of ribosomal cistrons[2]. Meanwhile, it was reported that the amount of r-DNA in liver and tail muscle of *Xenopus* larvae remained unchanged during thyroxine-induced metamorphosis[8].

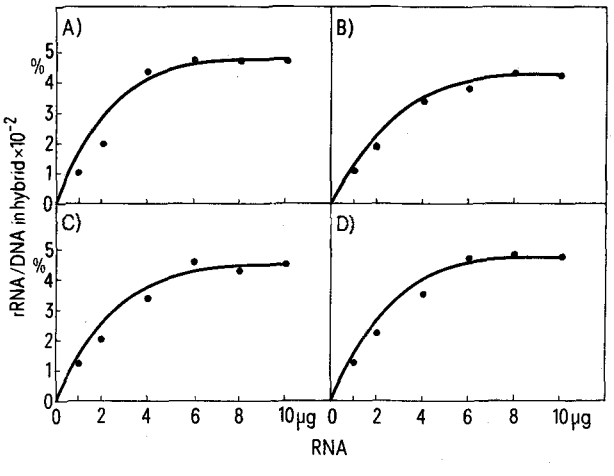
In the study reported here, fetal mouse liver was investigated with respect to the amount of ribosomal cistrons because there is also a great deal of variation in the number and size of nucleoli according to the developmental stage[9].

Materials and methods. Timed pregnancies in virgin female mice (NMRI strain, Ivanovas, Kissleg, W. Germany) were obtained by hormone priming, according to the method of SOUTHARD et al.[10]. The females were allowed to mate for 24 h. For the present set of studies, the next day was designated as the first day of gestation. The tissues from the liver region of 11-day-old embryos were removed into cold saline. Nuclei were isolated from these as well as from older liver tissues, according to the method of HOGEBOOM[11]. Deproteinization and further purification of DNA was carried out as previously described[2]. DNA was denaturated in 0.01 X standard saline citrate (0.15 *M* NaCl, 0.015 *M* sodium citrate) immediately before use in hybridization by heating in boiling water bath and cooling it rapidly in ice. ^{3}H-uridine labelled r-RNA was isolated from the ribosomal fraction of a subculture of mouse embryo cells. It was purified on a methylated-albumin-celite column as proved by gel-electrophoresis. Its specific radioactivity was 80,000 cpm/μg RNA. The RNA/DNA-hybridization was done in solution, as described by JEANTEUR and ATTARDI[12], by a modified technique as communicated previously[2]. From the reaction mixture, the unreacted RNA was removed by ribonuclease treatment followed by chromatographic separation on Sephadex G 75. Further separation of hybrids from reassociated DNA molecules was achieved by passing through nitrocellulose filters. The percentage recovery of hybridized DNA collected on the filters was calculated.

Results and discussion. The Figures show saturation curves for r-RNA/DNA in various combinations. In these experiments, increasing amounts of ^{3}H-labelled r-RNA from mouse cells were annealed with a constant amount of DNA from livers in different developmental stages. DNA from liver tissues from both 11- or 17-day-old fetal mice, known to consist of hematopoetic cells, were compared with those from newborn (2 days) or adult mice, as they have been reported to be comprised of mainly hepatocytes[13]. In all cases, a saturation level of about 0.045% of r-RNA in RNA/DNA-hybrid was attained.

Concerning hematopoetic cells, the present results are contradictory to those reported in the case of regenerating lens[7] or of liver cells in response to L-triiodothyronine[6]. Nevertheless, proerythroblasts as the predominant cell type (about 80%) among hematopoetic cells during days 10 to 12 of gestation[14] are known to contain enlarged and proliferated nucleoli[9]. This presents one criterion by which such liver cells can be distinguished very clearly from liver cells of 17 day embryos. Therefore, this morphological and biological difference, as regards to the rate of RNA synthesis, is not caused by an amplification of ribosomal cistrons in hematopoetic stem cells.

So far as the regenerating lens of *Triturus* is concerned, it seems to be the sole somatic tissue whose enhanced r-RNA synthesis[15] is assumed to be related to the process



Hybridization of liver DNA from 11-day-old fetal mice (A), 17-day-old fetal mice (B), newborn (2 days old) (C) or adult mice (D) with increasing amounts of r-RNA. The scale on the ordinate represents the fraction of nuclear DNA complementary to r-RNA. Each point presents the average value of 2 separate experiments.

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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]).