Pentose shunt enzyme activities in the preimplantation mouse and rabbit embryo

Stage of development	6-Phosphogluconate dehydrogenase Activity in moles of NADP reduced per embryo per h × 10^{12} Mouse		Rabbit		Glucose 6-phosphate dehydrogenase Activity in moles of NADP reduced per embryo per h × 10^{12} Mouse[1]	Rabbit[3]	Protein content in ng per embryo Mouse[8]	Rabbit
Unfertilized	13.6 ± 0.7	(4)	246 ± 22	(6)	1390	263	27.8	100
Fertilized	12.3 ± 0.7	(5)	256 ± 38	(6)	1360	243	27.8	100
Day 2	11.0 ± 1.1	(5)	251 ± 70	(6)	1510	263	26.1	100
Day 3	28.8 ± 3.2	(5)	259 ± 43	(6)	1400	215	23.4	100
Day 4	57.7 ± 1.1	(5)	338 ± 25	(6)	735	256	22.2	–
Day 5	61.3 ± 5.2	(4)	573 ± 68	(6)	190	812	21.9	1307
Day 6	–		3088 ± 517	(6)	–	1612	–	6235

Values for 6-phosphogluconate dehydrogenase are means ± S.E.M. The number of determinations is in parentheses. Protein content for the first 3 days of development in the rabbit is estimated to be about 100 ng based on the volume of the rabbit embryo which is about 3.5 times the volume of the mouse embryo in the early stages. Protein content of day 5 and day 6 rabbit embryos was determined by the method of LOWRY, ROSEBROUGH, FARR and RANDALL[9].

In the mouse embryo 6PGD activity is very low during the preimplantation period and is about $^1/_{100}$ the G6PD activity during the first 2 days of development. However, the pattern of change for 6PGD is opposite that found for G6PD; the former rises and the latter decreases during the last half of the preimplantation period so that their activity levels are approaching one another at the time of implantation. The 6PGD specific activity actually increases in the mouse embryo during the preimplantation period.

The low level of activity of 6PGD during the preimplantation period, particularly during the first 2 or 3 days of development, suggests that this enzyme is more important than G6PD in regulating the activity of the pentose shunt in the mouse. The high level of G6PD in the mouse embryo may represent enzyme present in the oocyte before ovulation and thus may reflect requirements for this enzyme during the preovulatory phase of oocyte development rather than during postovulatory embryo development[10].

Zusammenfassung. Im frisch ovulierten Ei der weissen Maus beträgt die Aktivität der 6-Phosphoglukonat-Dehydrogenase (6PGD) nur etwa $^1/_{100}$ derjenigen der Glukose-6-Phosphat-Dehydrogenase. Beide Enzyme zeigen ähnliche Aktivitäten bei Kaninchen-Embryonen vor der Implantation.

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Nuclear Pore Flow Rates of Ribonucleic Acids in the Mature Rat Hepatocyte

In a previous note we described the transport efficiency of a nuclear envelope pore complex with respect to the nucleocytoplasmic exchange of macromolecules and particles by the nuclear pore flow rate (NPFR), i.e. the total mass or number of molecules of a certain substance which is transferred through an average pore per minute[1]. Knowing the structural characteristics of the nuclear envelope of a given cell, such values can be calculated, e.g., for the unidirectional flow of nucleocytoplasmically migrating RNAs from theoretically different situations: 1. NPFR of cytoplasmic RNAs can be determined from measurements of the cytoplasmic increase of the RNAs during a specific interval of cell cycle or differentiation. In such calculations, non-nuclear RNA synthesis as well as the RNA-degradation have to be taken into account. As an example of this type of calculation, the NPFR values for distinct time spans of the amphibian oogenesis have been determined[1,2]. 2. In exponentially growing cell populations NPFRs of RNAs can be computed from the average content of cytoplasmic RNAs and from the half-life times of the type of RNA in question[1]. Again, such values have to be corrected for non-nuclear RNA-synthetic processes. 3. For cells which live in steady state conditions, such as many highly differentiated ones, it is possible to calculate the NPFR values from determinations of the pool sizes of cytoplasmic RNAs and their half-lives.

A calculation of the latter type has now been made for the average liver cell of adult rat. Combination of turnover and pool size data of cytoplasmic RNAs with

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Nuclear pore flow rates for ribosomal and transfer RNAs per average hepatocyte of 160–190 g albino rats (Wistar) as calculated from the steady state assumption NPFR = synthetic rate per average hepatocyte/mean number of pores per nucleus and synthetic rate = $1n\,2 \times$ (cytoplasmic concentration of product) $\times (t_{1/2} \times \min)^{-1}$

Electron microscopic method used for determining nuclear pore frequencies	Mean pore frequency per μm^2 nuclear surface	Mean nuclear diameter (μm)	Total No. of nuclear pores/average nucleus	$NPFR_{N\rightarrow C}$ (10–12 μg rRNA/ pore/min)	$NPFR_{N\rightarrow C}$ (rRNA equivalent to 1 ribosome/pore/ min)	$NPFR_{N\rightarrow C}$ (molecules rRNA/ pore/min) [d]	$NPFR_{N\rightarrow C}$ (molecules tRNA/ pore/min)
Negative staining of isolated nuclear envelope pieces	35.8	8.02 [a]	7.3×10^3	0.45	0.113	0.23	1.5
Glutaraldehyde-OsO_4-fixation, ethanol dehydration, epoxy resin embedding, thin sectioning	16.3	8.04 [b]	3.3×10^3	1.00	0.250	0.51	3.3
Freeze-etching of aldehyde-prestabilized tissue	14.1	8.10 [c]	2.9×10^3	1.14	0.286	0.57	3.8

Average DNA content of the nuclei was 9.1×10^{-12} g. The number of ribosomes per average cell was calculated as 7.6×10^6 from RNA/DNA ratios and the assumption that 90% of the cellular RNA is constituted by rRNA (compare[7–9]). A half-life of rRNA of 106 h was assumed[10–13]. The corresponding data for tRNA were taken from the work of WILSON and QUINCEY[9,13] (see there for further references). rRNA equivalent to one ribosome means the sum of 28s + 18s + 5s RNA present in a ribosome. Index N(ucleus) → C(ytoplasm) gives direction of the flow considered. [a] Values obtained from nuclei isolated with the procedure described elsewhere. [b] Values obtained from 1 μm thick sections through the same blocks as used for EM work. [c] Values obtained from 10 μm freeze-sections (WKF cryotome) of fixed tissue as used for the EM freeze-etch work. [d] Assuming a mean molecular weight of 1.2×10^6 D.

the structural data of the nuclear envelopes in this tissue yielded the NPFR values for tRNA and rRNA as listed in the Table. Since the different electron microscopic preparation techniques result in more or less divergent pore frequency values (FRANKE[3,4]; for detailed discussion of this problem see KARTENBECK et al.[5]) and since no decision on the in vivo state can be made at the moment, all the 3 basic techniques have been considered in parallel. As a consequence of the fact that the mature rat liver represents a mosaic of binucleate and mononucleate cells, as well as different steps in polyploidy, all values listed refer to the abstract but useful term of the 'average hepatocyte'. This describes the cellular portion belonging to an average nucleus which was characterized in our material (rats of 160–190 g body weight) by a mean DNA content of 9.1 picograms. The values for rRNA given in the Table also reflect the total cellular RNA since rRNA constitutes 80–90% of total RNA in rat liver.

It is apparent from these data that the hepatocyte nuclear pores show a RNA transport rate which is slightly less than that of lampbrush stage *Xenopus laevis* oocytes[1,2]. It is also below that of HeLa cells by a factor of about 4 (when comparing with values calculated with data from negative stain preparations of isolated envelope pieces) or 7 (when using the structural data obtained from freeze-etch work). The NPFR values in all these cell types mentioned, however, are much lower, for instance, than that of the macronucleus of the ciliate *Tetrahymena pyriformis* GL during exponential growth. In this cell, the average macronuclear pore conveys 45.8×10^{-12} μg as calculated on the basis of data obtained from negatively stained isolated membranes, and nearly 200×10^{-12} μg as calculated using freeze-etch data given by SPETH and WUNDERLICH[6]. Thus, the RNA transport capacity of a nuclear pore appears to be more than hundredfold higher in *Tetrahymena* than in a hepatocyte.

Zusammenfassung. Aus den Fliessgleichgewichts-Werten (mittlere Kernporenzahl und RNA-Synthesegeschwindigkeiten) der ausdifferenzierten Rattenleberzelle wurde die Kernporen-Durchflussrate (NPFR) für ribosomale und transfer RNA berechnet. Diese Hepatocytenwerte werden mit den entsprechenden RNA-Transportleistungen der Kernporenkomplexe anderer Zelltypen verglichen.

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The Influence of Several Sterols on the Conversion of β-Sitosterol into Cholesterol in the Cockroach

Although sterols are important for them, insects do not possess the enzymic system necessary for the synthesis of sterols from acetate or mevalonate. Therefore, sterols are indispensable components in the insects' food. Carnivorous insects obtain cholesterol from their food and they are able to convert it into the moulting hormones α- and β-ecdysone. Plant-eating insects obtain β-sitosterol from their food and they can convert it into cholesterol,