FREE NUCLEOTIDES OF THE LIVER DURING ONTOGENESIS

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1. Introduction

The functional rhythm of the hepatic cell during embryonal life has certain characteristics related to the peculiarity of many metabolic processes such as the late onset of hepatic glycogenesis, the various energy sources utilized, and the accumulation of visible lipid deposits in the liver.

In birds, the termination of ontogenesis is characterized by marked changes in hepatic cell metabolism. In fact, an accumulation of lipid and glucose stores which are subsequently rapidly utilized may be observed [1, 2]. In particular, in the Gallus gallus embryo, large amounts of fats are accumulated from the fifteenth day of incubation [3–6].

Neoglycogenesis, evaluated enzymatically, reaches its maximum at about the 16th to 17th day and subsequently declines, while the so-called "physiological steatosis" of embryonal liver rises [7].

Besides our cytochemical research [8] on hepatocyte differentiation and the metabolic needs of hatching, we have analyzed the lipid and nucleic acid contents of embryo liver [9]. In this connection it seemed interesting to analyze the free nucleotide spectrum and to follow its possible variation in level, as well as to consider its significance in certain fundamental metabolic pathways.

2. Methods

Eggs of the white Leghorn strain were incubated at 39°C. The livers were quickly removed at the 9th, 12th, 15th, 17th, 19th, and 21st day of incubation; they were subsequently frozen in liquid nitrogen, lyophilized and free nucleotides extracted and analyzed as previously described [10, 11].

In order to eliminate varying factors such as hydration and fat accumulation, the level of nucleotides refer to 100 g dry wt. of defatted tissue.

Fifteen free nucleotides may be extracted from the embryonal liver. They correspond qualitatively to those found in muscle and brain; they are present quantitatively in concentrations associated with other organs [11, 12].

3. Results

Table 1 shows the average content of total and single nucleotides. The percentage of total free nucleotides decreases from the beginning of our observations up to hatching. This decrease is especially marked for nucleoside triphosphates which represent about 50% of the total nucleotides from the 9th to 12th day and subsequently falls to 11% at hatching (table 2). The decrease in all the triphosphate fractions has been measured (table 1) by analysis of individual nucleotides; in particular CTP was present in measurable amounts on only the 9th and 12th days, whereas at later periods only traces were found.

A decrease in UDPglucoses was noted for the entire period.

Analysis of monophosphate nucleosides revealed that NAD, AMP and GMP levels rose sharply until hatching, whereas only slight oscillations in UMP, IMP and, after 12th day, CMP levels were shown.

The percentage of nucleotides of the same base is

Table 1
Free nucleotides in liver during ontogenesis.

Days	9th[a]	12th[a]	15th[a]	17th[a]	19th[a]	21st[a]
CMP	140[b]	74	59	57	58	66
NAD	130	149	187	191	238	245
AMP	216	258	312	326	357	425
GMP	57	74	71	60	83	97
UMP	143	100	149	146	151	127
IMP	105	73	108	108	111	93
ADP	431	415	392	408	389	245
UDPAG	223	252	183	150	145	121
UDPG	120	125	87	52	74	68
GDP	119	186	124	168	158	148
CTP	50	74	–	–	–	–
UDP	49	60	34	26	32	42
ATP	884	974	486	571	331	115
GTP	155	215	78	96	81	48
UTP	397	515	133	95	102	52
ΣNt	3219	3544	2403	2454	2310	1892
ATP/ADP	2.0	2.3	1.2	1.3	0.8	0.4
GTP/GDP	1.3	1.1	0.6	0.5	0.5	0.3
UTP/UDP	8.0	8.5	3.8	3.6	3.1	1.2
Number of individual livers	130	70	40	15	12	16
dry wt per liver (mg)	5.0	14.0	64.2	102.6	170.6	251.2
lipids[c] /dry wt (%)	6.3	18.5	24.1	31.7	45.1	50.7

[a] These values refer to the defatted dry weight.
[b] Results expressed as μmoles/100 g dry wt. ΣNt denotes total nucleotides (μmoles/100 g dry wt.).
[c] These values are taken from Romanoff p. 69 [20].

Table 2
Nucleoside triphosphates levels compared with total free nucleotides.

Days	9th	12th	15th	17th	19th	21st
NTP	1486	1778	697	788	514	215
Free Nt	3219	3544	2403	2454	2310	1892
NTP/free Nt	0.46	0.50	0.28	0.31	0.22	0.11

NTP, total nucleoside triphosphates; free Nt, total free nucleotides.

Table 3
Percentage of different nucleotides of the same base during ontogenesis.

Days	9th	12th	15th	17th	19th	21st
Adenine Nt* + IMP	50.7	48.4	53.9	57.5	51.3	46.3
Guanine Nt*	10.3	13.4	11.3	13.2	13.9	15.4
Uracil Nt*	28.9	29.6	24.4	19.1	21.8	21.7
Cytosine Nt*	5.9	4.2	2.4	2.3	2.5	3.5

* Nucleotide total.

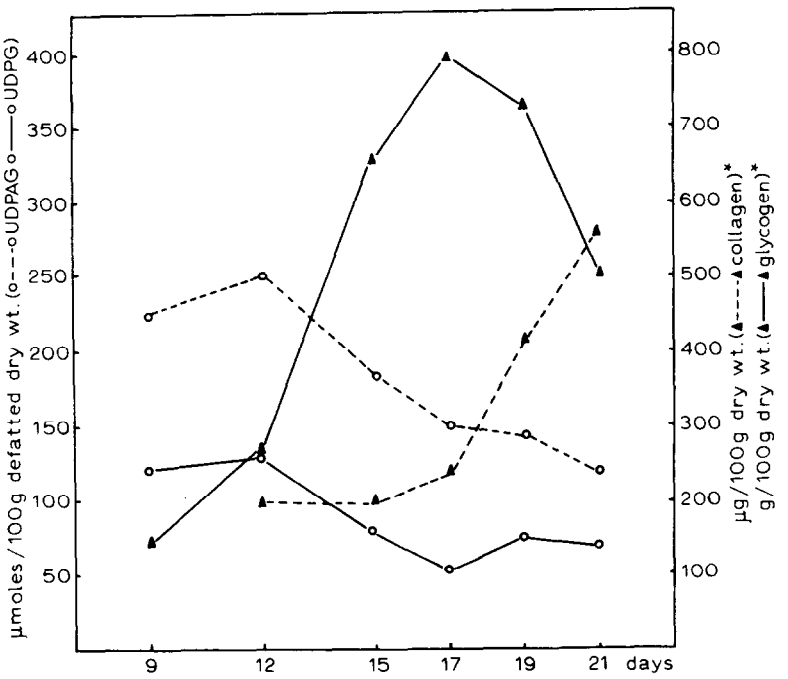

Fig. 1. Values of UDPAG and UDPG related with collagen and glycogen synthesis.
* These values are taken from Romanoff [20].

shown in table 3; during the entire period considered, the adenine nucleotides represented 50%, the guanine nucleotides increased from 10 to 15% and the uridine nucleotides decreased from 29 to 21%. The cytidine nucleotides consisted of only 2 to 5% of the total.

4. Discussion

The high percentage of adenine nucleotides found may be related to a variety of biological processes in which they are involved e.g. energy supply, both for differentiation and for the functional activity of the hepatocyte. The decrease in ATP and ATP/ADP ratio, observed from 15th day until hatching, is probably connected with a decrease in phosphorylation.

It is possible that the increase of AMP is correlated with variations of neoglycogenesis during the final incubation period; it is known that AMP regulates glycogen synthesis by accelerating the formation of pyruvic acid from glycogen [13]. The GMP increase also may be related to glycogenesis since GMP inhibits phosphoenolpyruvate formation from oxaloacetate [14].

As already shown in brain, the UDPglucose (UDPG) decrease in liver can also be correlated with an increased glycogen synthesis [15], until at least the 17th day (fig. 1).

The decrease of UDPacetylglucosamine (UDPAG) in liver and also in brain [10], is presumably related to collagen production which increases during the final period of incubation because of the production and strengthening of the supporting tissue network [15, 16].

The increase of NAD is probably connected with an increase in activity of liver mitochondria [17].

Phospholipid accumulation in liver [18] may be associated with the disappearance of CTP [19].

The rearrangement of hepatic metabolic functions accompanying birth therefore affects not only the relevant hepatocellular activities, such as glycogenesis, collagen synthesis and lipid metabolism, but also the metabolism of nucleotides connected with these processes.

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