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The Amount of Adenine Nucleotides and Glycolytic Intermediates in Erythrocytes, Liver and Muscle Tissue Correlated with the Body Weight (Age) in Wistar Rats

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ABSTRACT

The concentrations of adenine nucleotides in the liver and skeletal muscle increased during the maturation period (during the first 100 days of life) and remained fairly constant in adult rats. The concentration of adenine nucleotides in erythrocytes decreased with age.

The concentrations of hexose monophosphates increased with age in liver and muscle tissue and decreased with age in erythrocytes.

The observed changes of metabolites in the different tissues with increasing age of rats could not be explained by a changed water content.

INTRODUCTION

Information about the nature and magnitude of age dependent changes of tissue metabolites is scanty, and as concerns adenine nucleotides and glycolytic intermediates almost lacking. Thus a number of intracellular metabolites: adenine nucleotides (ATP, ADP, AMP), glucose 6-phosphate (G-6-P), fructose 6-phosphate (F-6-P), were determined in liver and skeletal muscle and in erythrocytes, respectively.

The amount of the metabolites was primarily correlated with the body weight. Statistically significant correlations were transformed into curves showing the relationship between the amount of tissue metabolite and age, where age was derived from the mean correlation curve between body weight and age for the type of rat used.

MATERIAL

White male rats of Wistar strain were used. During the experiments all rats were kept single in metabolic cages and daily measurements of body weight and the amount of food and water consumed were made. They were fed on a standard diet supplemented with vitamins, with a caloric content of 51 per cent carbohydrate, 32 per cent fat and 17 per cent protein. The animals received food ad libitum and they had free access to water. All rats were starved 24 hours

prior to sacrifice.

The rats were divided in two groups: 49 animals were used for the determination of intracellular metabolites in liver and skeletal muscle and in erythrocytes. 17 animals were used for the measurement of the apparent cell water in the same type of cells.

METHODS

Ether anaesthesia and removal of tissue

Rapid, light ether anaesthesia associated with minimal disturbance of the rats was achieved. The liver was exposed through a mid-line, abdominal incision. Small pieces of liver were removed using precooled stainless steel tongs which were immersed in liquid nitrogen. Tissue from M. quadriceps femoris was taken by the same sampling procedure. 25-50 mg of liver and muscle tissue was obtained. The time required for the incision to the sampling varied between 15-30 seconds, on the average faster for the liver. Blood (about 1 ml) was collected from the heart by a syringe and immediately transferred into 5 ml of 6 % (v/v) ice cold perchloric acid.

In order to study the accuracy of the sampling procedure biopsies also were stored in vitro during certain time intervals before freezing the specimen in liquid nitrogen.

Extraction of tissue

The frozen tissue was prepared for analysis of the different substances according to Dale (5) except that liquid nitrogen was used instead of liquid oxygen and that the perchloric acid extract of the different tissues was brought to pH 6.5 instead of pH 3.5.

Chemical methods

ATP, ADP and AMP were determined according to Adam (1) by enzymatic methods and with spectrophotometric readings. The methods are not absolute specific for ATP, ADP and AMP as also other tri-, di- and mononucleotides, if present, may react. The error introduced in the actual values are probably of minor importance, however, as the amount of ATP in perchloric acid extracts of liver tissue determined by the method given above and controlled by the more specific hexokinase methods (17) did not differ significantly ($P>0.10$, $n=10$). G-6-P and F-6-P were determined enzymatically (14) and with fluorimetric readings.

Glycogen was converted to glucose (21) and the glucose concentration was determined with the glucose oxidase method of Hjelm and de Verdier (13). Haemoglobin was determined by the cyanomethaemoglobin method and haematocrit values were obtained with an International Micro-Capillary Centrifuge Model MB, International Equipment Company, Nedham, HTS, Mass., USA. The haematocrit values

were not corrected for trapped plasma (7).

The amount of "free-water" was estimated in liver and muscle tissues by means of dehydrating tissue pieces of approximately equal size to constant weight at a temperature of +105°C in a thermostat containing a water absorbing medium.

Calculations

The statistical method used for correlations between metabolites and body weight was piece-wise linear regression (8). The relationship between age and body weight established by the local breeder of the Wistar rats, was used to transform the experimentally obtained relationship between the content of metabolites and body weight into one between the content of the metabolites and age.

RESULTS AND DISCUSSION

1. Methodological aspects on the procedure for sampling of tissues

The quite considerable change of the concentration of a number of metabolites in liver biopsies within seconds is shown in Fig. 1.

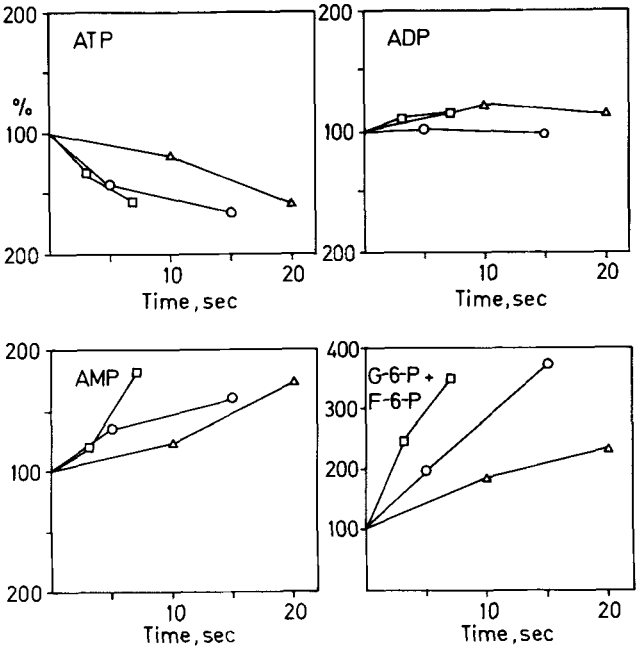


Fig. 1. Concentrations of ATP, ADP, AMP, G-6-P and F-6-P in liver biopsies removed using precooled stainless steel tongs (=100 %) and taken with knife and immersed in liquid nitrogen after 3,5, 7, 10, 15 and 20 seconds respectively.

The result stresses the importance of an accurate sampling technique of tissues with a rapid turnover of metabolites in order to obtain relevant information about the conditions in vivo. The inconsistency between values for some tissue metabolites in rats of comparative weights, compiles from the literature in Table 1, indicates that the sampling of tissues might not always have been accurate and that earlier results cannot directly be used as reference values.

Table 1. The average concentrations of adenine nucleotides in muscle tissue, liver tissue and erythrocytes in rats and some other species. (Range of body weight 100-200).

Tissue	ATP	ADP	AMP	ΣATP, ADP, AMP
Muscle tissue[1)]				
Imai, Riley & Berne (1964)	6.3	0.76	0.29	7.4
Pedersen & Sachs (1965)	4.8	-	-	-
Scopes & Newbold (1968)	4.5	1.3	0.11	5.9
Chaudry, Sayeed & Baue (1974)	3.6	0.6	0.07	4.3
Berne & Rubio (1974)	5.43	1.29	0.21	6.9
Present investigation	4.8	0.9	0.1	5.8
Liver tissue[1)]				
Maass & Timm (1964)	2.3	0.78	-	-
Kolousek, Jiracek, Zicha et al (1965)	3.2	1.71	0.57	5.4
Hems, Ross, Berry et al (1966)	2.5	0.94	0.21	3.7
Puddu, Caldarera & Marchetti (1967)	0.33	0.90	2.1	3.3
Chaudry, Sayeed & Baue (1974)	1.85	0.82	0.25	2.9
Hirasawa, Chaudry & Baue (1978)	2.47	0.73	0.14	3.3
Ozawa et al (1981)	2.38	0.55	0.14	3.1
Present investigation	3.0	1.2	0.4	4.6
Erythrocytes[1) 2)]				
Kolousek, Jiracek, Zicha et al (1965)	1.57	0.17	0.05	1.8
Present investigation	1.9	0.4	-	-

[1)] Moles x 10^{-6} per ml of erythrocytes or per gram of wet weight of liver or muscle tissue

[2)] The average haematocrit value assumed to be 40 %

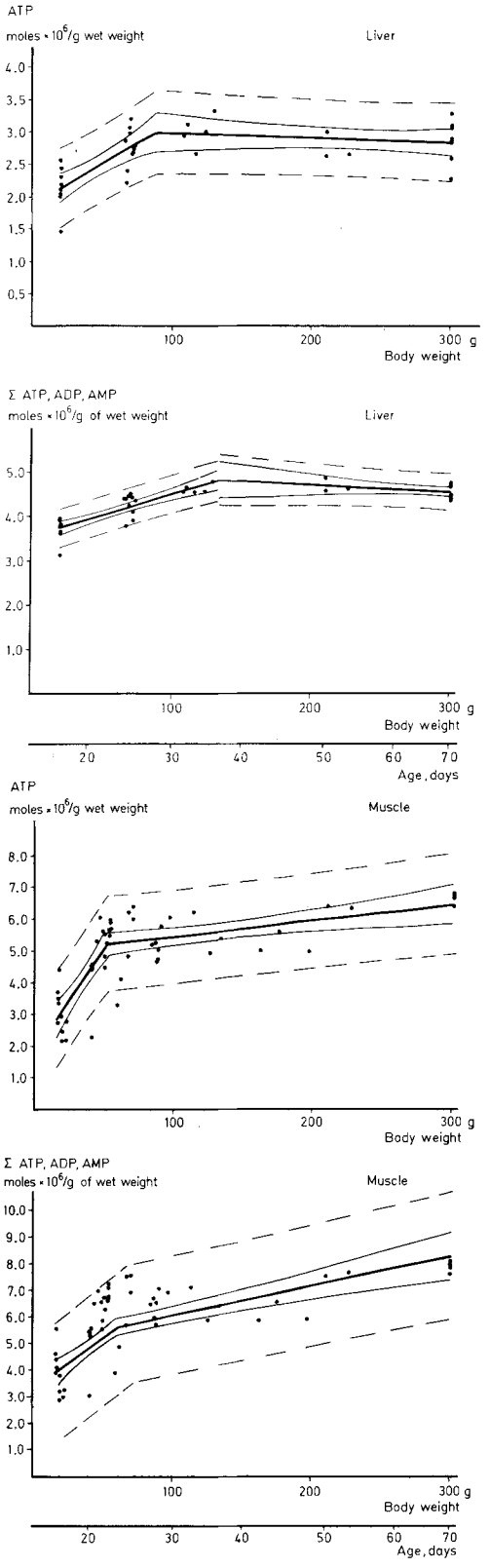
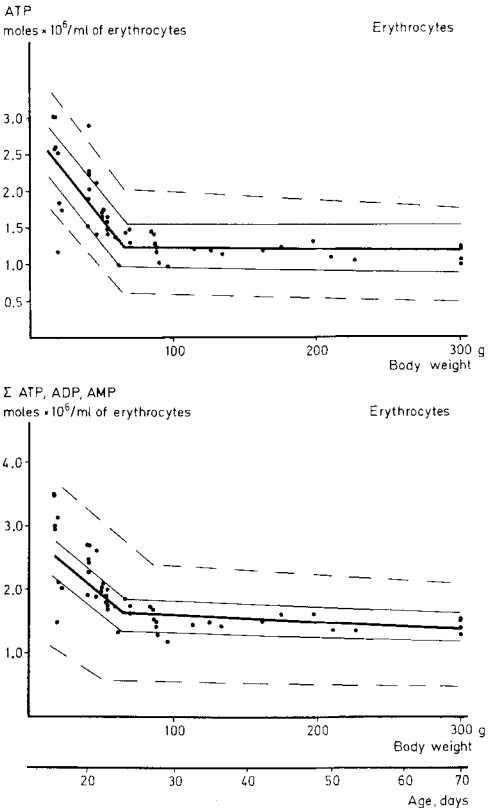


Fig. 2. Concentrations of ATP and the sum of ATP, ADP and AMP in liver, skeletal muscle and erythrocytes correlated with body weight (age). Regression line with 95 % confidence interval and 95 % tolerance limit for the measurements is indicated.



2. The concentration of tissue metabolites correlated with body weight (age)

2.1. Adenine nucleotides in muscle and liver tissue and in erythrocytes

The concentration of ATP in the liver increased significantly during the maturation period, i.e. during the first 100 days of life, and remained fairly constant in adult rats (Fig. 2). Preliminary studies in rats showed an increased number of mitochondria in liver tissue from animals with a body weight of 400 g compared to 60 g. The observed increase of the content of ATP in the liver of maturing rats may accordingly be due to an increased number of mitochondria. The observed changes of ADP and AMP and of the sum of adenine nucleotides with age (Fig. 2) could accordingly be secondary to keeping the over all equilibrium of the adenylate kinase reaction, $(ATP \times AMP)/(ADP)^2$ at a constant level.

The concentration of adenine nucleotides in muscle tissue changed with age in a similar way as in the liver. It is, however, not known if the number of mitochondria in muscle tissue varies with age. The situation is also complicated by the fact that muscle tissue is composed of muscle fibres of different types, which might contain the same or a different number of mitochondria per unit volume of tissue. Thus a change of the relative frequency of the types of muscle fibres with age might explain the observed change of the over all content of ATP. Work is in progress to clarify this problem.

The concentration of adenine nucleotides in erythrocytes decreased slowly during the maturation period. In human erythrocytes HbF has a higher affinity to 2,3 diphosphoglycerate than HbA (6). Intact erythrocytes in neonates contain about twice as much of the phosphocompound as in adults to assure the same oxygen release capacity of erythrocytes in neonates and adults (11, 12). The situation might be analogous for the binding of ATP to a fetal and adult type of rat haemoglobin. Then the decreasing content of ATP in erythrocytes from young rats could be explained by a concomitant decrease of fetal rat haemoglobin. However, little is known about rat haemoglobins at present and especially about the binding of organic phosphocompounds to the molecule. More experimental work is necessary to clarify the situation. It does not seem likely, however, that the decreased content of ATP is related to a changed mean age of the circulating erythrocyte population (cf. 12).

There was a trend for the apparent equilibrium constant, K, for the adenylate kinase reaction, $(ATP \times AMP)/(ADP)^2$ to decrease with age in liver and muscle tissue, whereas the energy charge of the adenylate pool calculated according to Atkinson (2): $(ATP + 0.5\ ADP)/(ATP + ADP + AMP)$ stayed constant. No obvious changes of these two parameters with age could be observed in erythrocytes (Fig. 3).

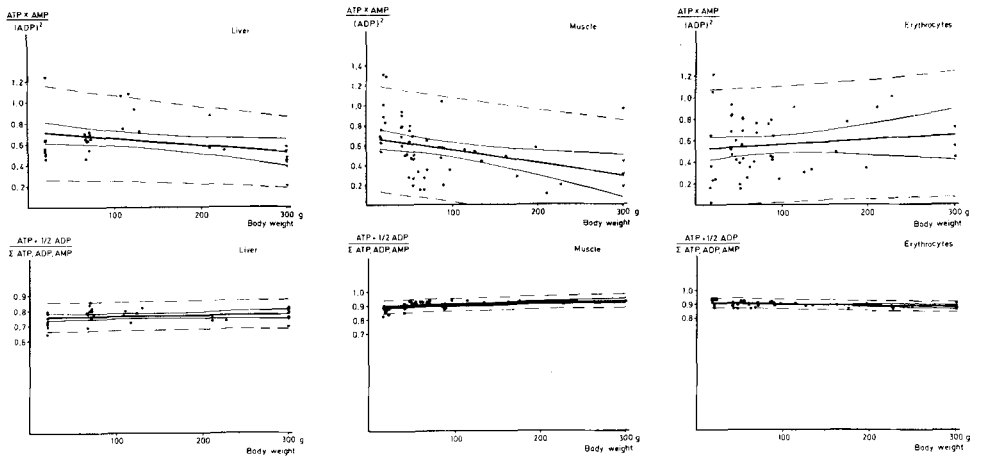


Fig. 3. The apparent equilibrium constant, K, for the adenylate kinase reaction (ATP x AMP)/(ADP)^2 and the energy charge of the adenylate pool in liver, skeletal muscle and erythrocytes correlated with body weight (age).

The total variance of ATP, ADP and AMP and of the apparent equilibrium constant, K, around the regression lines against age showed a considerable variance, in the order of ± 10 to ± 15 % in all tissues studied (Fig. 2 and 3). The total variance around the regression line for the energy charge against age is considerably less and in the order of ± 5 % (Fig. 3). A major part of this variance could be explained by factors related to the handling of the specimens and the analytical procedure. If this assumption is correct the inter-individual (biological) variance of the energy charge must be extremely low. It also follows that the considerable inter-individual variance observed for the individual adenine nucleotides could reflect a homeostatic mechanism by which the demand of keeping the energy charge within a narrow absolute limit is linked to a varying intermediary energy metabolism, adjusted to meet individual demands.

It should, however, be pointed out that the energy charge is a lumped parameter in liver and kidney tissue as both liver and muscle cells represent multicompartment systems with adenine nucleotides present both in mitochondria and cytoplasma and related to the cell membrane.

2.2. Hexose monophosphates in muscle and liver tissue and in erythrocytes.

In Fig. 4 the concentration of the hexosemonophosphates glucose-6-phosphate and fructose-6-phosphate are correlated with body weight. Evidently there is an increase of the metabolites in both muscle and liver tissue, most pronounced in young rats. How this findings should be explained is uncertain. Though the number of measurements in erythrocytes is limited the result indicates that there is a decrease of the amount of the hexosemonophosphates, also in young animals. This might be related to the different binding of the compound to a fetal and

adult type of rat haemoglobin.

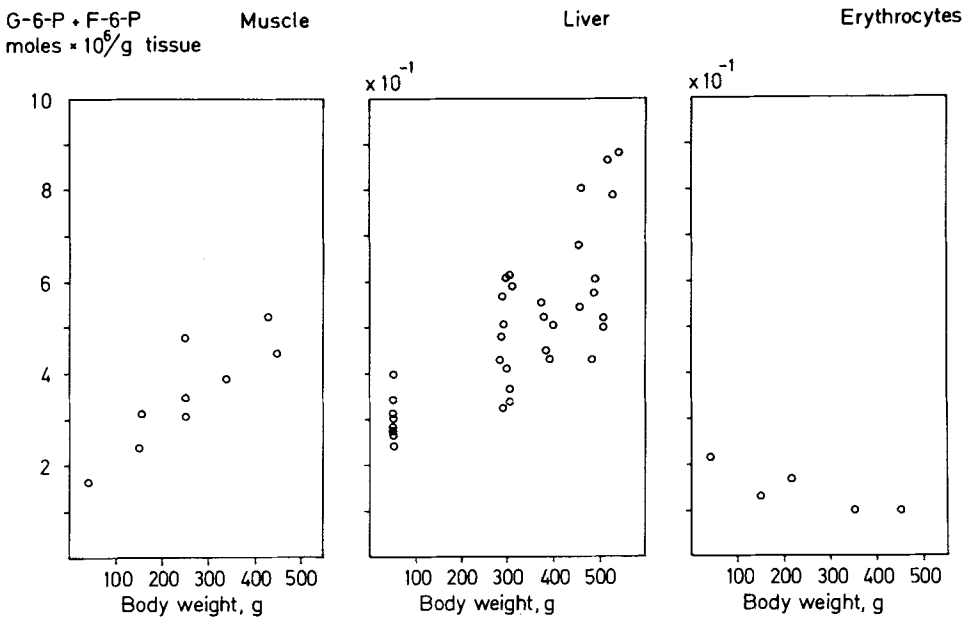


Fig. 4. Concentration of G-6-P and F-6-P in liver, skeletal muscle and erythrocytes correlated with body weight (age).

2.3. Glycogen in liver tissue

The amount of liver glycogen has been correlated with body weight and the age of the animal in Fig. 5. There is a pronounced increase of glycogen during the first seventy days of life and then a decrease of the compound. There is no obvious explanation for the finding. However, it seems of importance to consider this result in future experimental situations where liver glycogen is determined either in rats of different age or during longitudinal studies.

2.4. Water in muscle and liver tissue and in erythrocytes

The content of water in the three types of cells investigated has been determined and correlated to the body weight or age. It appears from Fig. 6 and 7 that the small changes observed are not at all of such a magnitude to explain the changes of metabolites as a result of a changed water content of the investigated cell types.

2.5. Other factors influencing the amount of metabolites in the tissue investigated

Other factors than those discussed above might be of importance to explain the present findings, e.g. the effect of light ether anaesthesia. This form of anaesthesia might in the absence of a blocking compound have released adrena-

line, activating the adenylate cylclase system and with secondary effects on the intermediary metabolism especially of the liver. It would be necessary to carry out additional work to elucidate this problem.

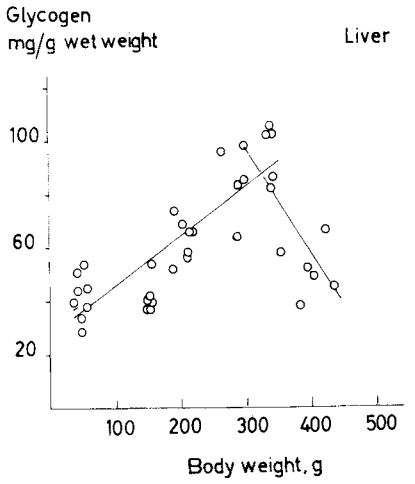


Fig. 5. The amount of glycogen in the liver correlated with body weight (age).

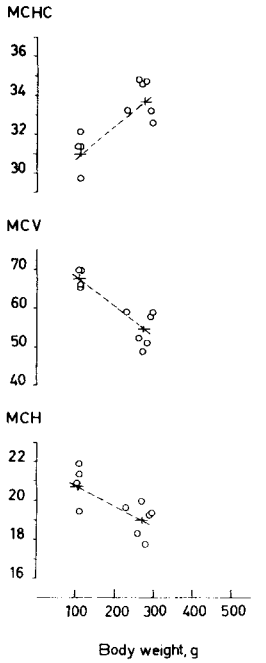


Fig. 6. MCHC, MCV and MCH in erythrocytes correlated with body weight (age).

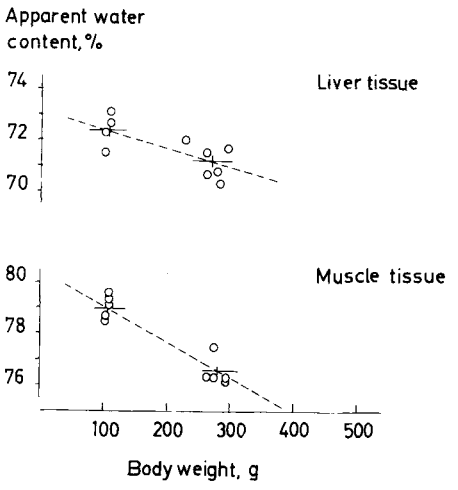


Fig. 7. The apparent water content of liver and muscle tissue correlated with body weight (age).

ACKNOWLEDGEMENT

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