# CHANGES IN FREE ADENOSINE TRI-, DI AND MONOPHOSPHATE CONCENTRATIONS IN THE BRAIN OF WHITE RATS, FED NORMAL AND RICH IN PROTEINS DIETS IN EXPERIMENTAL MANGANESE TREATMENT

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Adenosine phosphates participate in numerous biological processes in their capacity of energy releasing mediators, sources of electrons or co-factors (10, 12, 17). A direct or feedback interrelation exists between their concentration and the activity of a number of enzyme systems (14). The synthesis of adenosine phosphates is accomplished within the mitochondria (11).

The continuous administration of large manganese doses leads to some changes in the structure of brain mitochondria, namely: vacuolation, fragmentation and alterations of matrix density (8). The brain ATPase activity increases (7). Many authors report the formation of complexes between adenosine phosphates and bivalent metal ions, in vitro. Manganese yields $MnATP^{2+}$ with a considerable stability at pH=7.0 (16); $MnADP^{-}$ and $MnAMP^{-}$ (15).

We were successful in establishing that rich in proteins rations exert a favourable effect on the changes brought about by manganese (1). Manganese concentration in the blood and organs is lower, the concentration of free amino acids — higher, and the activity of some enzyme systems is inhibited to a lesser degree than in manganese treated animals, fed normal diets (18 per cent B — in cal).

The issue of the changes in brain adenosine phosphate content, taking place under the influence of toxic doses manganese against the background of a rich protein diet has not been clarified thus far which led us to undertake the present study.

## Material and Methods

Investigations were carried out on 128 male white rats, weighing 120± 20 g in the average, distributed in groups as shown in Table 1. Poisoning was performed every other day, two hours before meal, per os, with 10.8 per cent $MnCl_2.4H_2O$ solution, at dose 150 mg $Mn^{2+}$/kg body weight, for a period of 60 days. The concentrations of the adenosine phosphates under study were determined at 30 and 60 days, according to the method of Kornasky (13), as modified by T. S. Ivanova and co-authors (3), using the tests of Böhringer, and calculated in nmol/g fresh tissue after Martin (14).

Table 1

**The Distribution of Animals by Groups and Rations**

| Groups | B (%) in cal | Addition | Number | | |
|---|---|---|---|---|---|
| | | | controls | poisoned | total |
| I | 18 | — | 32 | 32 | 64 |
| II | 35 | 0.44 g cassein | 32 | 32 | 64 |

## Results and Discussion

Of the total free nucleotides' content in the brain of control animals, maintained on a normal diet, ATP amounts to 28 per cent, ADP — 16.5 per cent and AMP — 8.5 per cent (Table 2). which is in line with the re-

Table 2

**Concentration of Free Adenosine Phosphates (nmol/g fresh tissue)**

| Index | 30th day | | p | 60th day | | p |
|---|---|---|---|---|---|---|
| | controls | poisoned | | controls | poisoned | |
| ATP | I 114.80±5.20<br>II 384.20±42.50<br>p I/II 0.01 | 57.20±10.40<br>89.40±8.30<br><0.05 | 0.001<br>0.001 | 111.20±25.10<br>311.70±28.50<br>0.01 | 123.60±15.20<br>80.60±11.30<br><0.05 | >0.05<br>0.001 |
| ADP | I 66.06±7.75<br>II 70.47±1.67<br>p I/II >0.05 | 42.30±4.65<br>70.72±1.75<br>0.01 | 0.01<br>0.05 | 66.57±7.23<br>76.14±4.83<br>>0.05 | 76.47±5.79<br>67.20±10.50<br>>0.05 | >0.05<br>>0.05 |
| AMP | I 34.87±6.14<br>II 32.25±2.35<br>p I/II >0.05 | 17.93±1.58<br>14.71±1.56<br>>0.05 | 0.001<br>0.001 | 32.43±4.52<br>44.01±3.44<br><0.05 | 71.36±6.65<br>39.71±7.53<br>0.01 | 0.01<br>0.05 |

sults submitted by Ivanova and co-authors (3). In the brain of the control animals, maintained on a rich protein diet, the ADP and AMP content was 1—2 per cent higher, while ATP — 87 per cent, i. e. three times higher than the figures reported by Ivanova. ATP is a regulator of the glutamic acid metabolism (2), and its elevated concentration may be explained by the considerable glutamic acid amount (about 1 g), daily introduced with casein.

About the 30th day, the free ATP, ADP and AMP concentrations in the brain of the poisoned animals fed a normal diet were reduced with almost 50 per cent. Among the poisoned animals, maintained on a rich in proteins regimen, the fall of AMP was of the same order, ADP was unchanged, while ATP was substantially reduced (Table 2). Within 60 days, the content of

free adenosine phosphates in the brain of poisoned animals on normal diet increased up to, and above the starting level, rather considerably for AMP (Table 2). In the brain of poisoned animals on rich protein diet, the ATP and ADP concentrations established at 30 days were preserved unchanged. AMP increased up to 90 per cent relative to controls.

The fall of free adenosine phosphates in the brain of poisoned animals, maintained on standard diets (18 per cent in cal), was most likely due to the prompt binding and Mn chelates formation, as well as to the as yet insufficient increase in the activity of $K^+$, $Na^+$, $Ca^+$ and $Mg^{2+}$-brain phosphatases. Along with that, the toxic manganese doses brought about a quick lowering of the concentrations of free brain amino acids which exert influence on the synthesis of adenosine phosphates (9, 18). It was established that under the effect of manganese, pyridoxal 5'-phosphate, respectively vitamin $B_6$, were also reduced (4, 6). Thus, the reduced concentration of ATP and ADP may be also explained.

Normalization of ATP and ADP at the end of the experiment is probably due to the enhanced ATPase activity, and to the different synthesis routes of adenosine phosphates. It is presumable that the substantial increase of AMP is the result of prompt ATP and ADP splitting (5), indispensable for the maintaining of a normal balance between them. Whenever glutamic acid concentration in the brain of poisoned rats decreases to a substantial degree, 2-oxoglutarate is probably included as a substrate, with predomination of the AMP synthesis (20). Similar conjectures may be also made insofar as changes in ATP and AMP in the brain of poisoned animals, fed diets enriched with casein, are concerned. The rich in proteins diet maintains the fall of free amino acids in the brain and glutamic acid to 74 per cent in relation to controls (1a). The relative proportion of the adenosine phosphates under study remains close to the normal distribution.

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## ИЗМЕНЕНИЯ КОНЦЕНТРАЦИЙ АДЕНОЗИН ТРИ-, ДИ- И МОНОФОСФАТОВ В МОЗГУ БЕЛЫХ КРЫС, ПОЛУЧАЮЩИХ ПИЩУ С НОРМАЛЬНЫМ И БОГАТЫМ БЕЛКАМИ РАЦИОНОМ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ВОЗДЕЙСТВИИ МАРГАНЦЕМ

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РЕЗЮМЕ

Концентрации свободных АТФ, АДФ и АМФ в мозгу имеют большое значение для нормального течения ряда метаболитных процессов.

На 30-й день, под влиянием 150 мг/кг веса введенного через рот $Mn^{2+}$, устанавливается снижение приблизительно на 50% исследованных аденозин фосфатов. На 60-й день их концентрации увеличиваются до и выше значений у контрольных животных. Самое значительное увеличение отмечается для АМФ. У животных находящихся на 35% богатом по кал. рационе, обогащенном прибавлением 0,44 г казеина в день, изменения концентраций слабее и аденозинфосфаты сохраняют пропорциональность распределения, близкое к нормальному.