Rhythmic variations in total amino acids and AAT and AlAT activities in the slug, *Laevicaulis alte*, were correlated to the animal's locomotor activity[5]. In the present work, AlAT activity is 2fold higher than AAT in the nervous system and coxal leg muscle homogenates of the cockroach. This suggests that transamination of alanine is greater in the tissues. Besides, AAT and AlAT activities were higher in muscle than in the nervous system. Higher levels of aminotransferases in the muscle suggest that, in association with motor activity, the tissue may show facultative energy metabolism. It is probable that in cockroaches the higher levels in AlAT activity are coupled with energy metabolism of the animal.

Table 2. De Ritis Quotient (AAT/AlAT)

	Time of day in h						
	8.00	12.00	16.00	20.00	0.00	4.00	Mean AAT/ mean AlAT
NS	0.39	0.51	0.48	0.35	0.51	0.499	0.46
MS	0.45	0.4	0.44	0.43	0.51	0.46	0.45

NS, Nervous system; MS, coxal leg muscle.

The feeding of amino acids into carbohydrate and lipid oxidation is mobilized by aminotransferases. Accelerated AAT and AlAT activities during night apparently reflect accelerated biological oxidations leading to energy supply. Associated with the activity phase of the animals[11], accelerated TCA cycle enzyme activities[12,13] were shown for scorpions. Hence, in cockroaches the enhanced AAT and AlAT activities during night dark hours probably relate to energy supply for overt locomotor activity of the animal.

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Biotin as a regulator of some haematic parameters and of DNA-content of the liver of old rats

F. Petrelli, S. Coderoni, P. Moretti, G. Marsili and M. Paparelli

Institute of General Physiology, University of Camerino (Italy), 13 June 1977

Summary. Biotin administration to old rats (28 months) causes in the blood an increase of ATP, glucose, triglycerides, alkaline phosphatase and a decrease of cholesterol and acid phosphatase; in the liver DNA and electrostatic interactions between DNA and histones are increased. Such parameters come within the values shown by adult rats.

In the whole blood of old rats, the ATP[1,2]-content is decreased; serum cholesterol[2,4] and alkaline phosphatase[2] are respectively increased and decreased; α- and γ-globulins are increased; for albumin the same amount both in old and young rats sera is observed. The accumulation of total globulins is not the consequence of a different rate of synthesis, but of a decreased rate of degradation[5]. The increase of the amount of α_1- and γ-globulins has been reported also by Vebel et al.[6,7] and Horne et al.[8]. Moreover the liver of aged rats shows a marked decrease in the ratio of arginine-rich to arginine-poor histone fractions. There is no significant age-associated change of total histone content of the liver[9,10]. The DNA of the old rat liver is decreased[2].

In view of the effect of biotin on protein synthesis, lipogenesis, glucose metabolism and oxidative phosphorylation[11-23], we have investigated the action of biotin on some haematic parameters (ATP, glucose, protein levels, total lipids, triglycerides, cholesterol, acid and alkaline phosphatase) and on the nucleic acid content and the electrostatic interactions between histone proteins and DNA of old rat liver.

Materials and methods. Female Sprague-Dawley rats aged 10 and 28 months were used. A group of old rats was treated every second day during with an aqueous solution of biotin (200 µg/100 g b.wt). Control groups of adult and old rats received an equal volume of saline.

24 h following the final injection and 12 h fasting, the animals were sacrificed by bleeding. The determinations of blood ATP and glucose were effected using respectively

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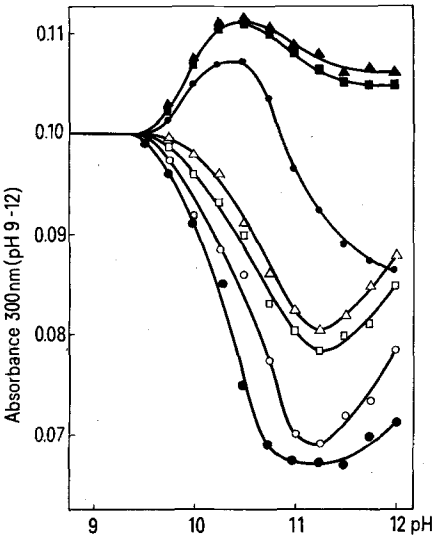
Table 1. Quantitative determinations of blood ATP and glucose, and of serum proteins, total lipids, triglycerides, cholesterol, alkaline and acid phosphatase

	Rats 10 months (nontreated)	28 months (nontreated)	28 months (treated)	p *a*	*b*	*c*
Blood ATP (mg/100 ml) (6)	29.00 ± 2.53	21.70 ± 3.54	27.70 ± 1.24	< 0.01	NS	< 0.01
Blood glucose (mg/100 ml) (6)	84.33 ± 5.85	65.00 ± 2.58	87.50 ± 11.59	< 0.001	NS	< 0.01
Serum proteins (g/100 ml) (6)	6.21 ± 0.08	7.90 ± 0.82	7.44 ± 0.56	< 0.001	< 0.001	NS
Serum total lipids (mg/100 ml) (6)	473.17 ± 30.73	470.00 ± 18.26	460.00 ± 73.94	NS	NS	NS
Serum triglycerides (mg/100 ml) (6)	103.33 ± 10.81	92.25 ± 14.38	140.00 ± 13.11	NS	< 0.001	< 0.001
Serum chlolesterol (mg/100 ml) (6)	78.33 ± 4.08	94.00 ± 5.89	72.00 ± 4.76	< 0.001	< 0.05	< 0.001
Serum alkaline phosphatase (mU/ml) (6)	72.33 ± 4.76	56.50 ± 9.40	72.50 ± 4.20	< 0.01	NS	< 0.02
Serum acid phosphatase (mU/ml) (6)	44.83 ± 4.07	77.50 ± 10.40	48.50 ± 15.11	< 0.001	NS	< 0.02

Mean values ± SD. Statistical significant, Student's t-test. *a* Differences between 28 months and 10 months nontreated rats; *b* differences between 28 months treated and 10 months nontreated rats; *c* differences between 28 months treated and nontreated rats. NS: Nonsignificant differences. The number of animals is given in parentheses.

the methods of Jaworek et al.[24] and Bergmeyer et al.[25]. The determinations of serum total proteins and lipids, triglycerides, cholesterol, acid and alkaline phosphatase were carried out according to Weichselbaum[26], Zöllner et al.[27], Wahletld[28], Allain et al.[29], Andersch et al.[30], Bessey et al.[31], respectively. DNA and RNA were extracted from the liver using the methods of Marmur[32] and Kay et al.[33], modified by Swindlehurst et al.[34].

The quantitative determinations of DNA and RNA were carried out with diphenylamine and orcinol respectively[35]. The preparation of the nuclei for the extraction of desoxyribonucleohistones (DNH) has been carried out according to the method described by Busch[36]. The extraction of DNH was carried out according to Bram et al.[37] method. The extracts were deproteinized by chloroform-isoamylic alcohol (24:1 v/v) according to Sevag et al.[38], in different conditions of ionic strength, i.e. in the absence or in the presence of 1 M $NaClO_4$. The RNA was removed from nucleoproteins by ribonuclease digestion. The DNH were determined spectrophotometrically according to the method described by Walker[39], taking in account that the absorption changes at 300 nm for DNA, DNH and histones when pH increases from 9 to 12.5.



Spectrophotometric titration curves at 300 nm of calf thymus DNA (A-grade, Calbiochem), ●–●; of DNH (deoxyribonucleohistones), ▲–▲ ●–● ■–■; and DNA, △–△ ○–○ □–□, obtained respectively from the liver of 10 months (nontreated), 28 months (nontreated) and 28 months (treated) rats. This titration is based on the variation of absorbance at 300 nm shown by DNA and DNH when the pH is increased from 9 to 12.

Results and discussion. From the results reported in table 1, one notes in old as compared to the adult untreated rats, a greater amount of total proteins and cholesterol in the serum, and a smaller amount of ATP in total blood: such results confirm previously published data[1–5]. Moreover, one notes a greater amount of acid phosphatase and a smaller amount of glucose and alkaline phosphatase; the total lipids and triglycerides are unchanged. The old rats given biotin show a significant increase of ATP, glucose, triglycerides and alkaline phosphatase, and a significant decrease of cholesterol and acid phosphatase, as compared to the untreated old rats; the total proteins and lipids are unvaried. The values of ATP, glucose, cholesterol acid and alkaline phosphatase of old rats treated with biotin come within the values shown by the adult rats. The results reported in table 2, in agreement to previously published data[2], show in old untreated rats, as compared to the adult ones, a smaller amount of liver DNA; as for RNA, no significant variation was found. Following biotin treatment, the DNA-values of old rats reached the values shown by adult rats. The titration of DNA and deoxyribonucleohistones (DNH) prepared according to the method of Bram and Ris[37] from the liver of old rats, un-

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Table 2. Quantitative determinations of DNA and RNA of rat liver

	Rats 10 months (nontreated)	28 months (nontreated)	28 months (treated)	p *a*	*b*	*c*
DNA (mg/g) (6)	2.731 ± 0.200	1.992 ± 0.138	2.957 ± 0.669	< 0.001	NS	< 0.01
RNA (mg/g) (6)	8.309 ± 0.728	8.027 ± 0.749	9.629 ± 1.579	NS	NS	NS

Mean values ± SD. Statistical significant, Student's t-test. *a* Differences between 28 months and 10 months nontreated rats; *b* differences between 28 months treated and 10 months nontreated rats; *c* differences between 28 months treated and nontreated rats. NS: Nonsignificant differences. The number of animals is given in parentheses.

treated or treated with biotin, and from the untreated adult rats, was carried out spectrophotometrically according to the Walker's method[39]. This is based on the variation of absorbance at 300 nm shown by DNA and DNH when the pH is increased from 9 to 12.5: such variations are due to the different ionization, correlated with the variation of the pH, of the cytosine and guanine groups as concerns the DNA, and of the phenolic groups of the tyrosine as concerns the histone proteins. The figure shows the pattern of the DNH and DNA extinction variations when pH increases from 9 to 12. The data, in accordance with previously published results[40,41], demonstrate in the DNH complex of the old untreated rats, as compared to the adult ones, a significant decrease of the electrostatic interactions between histone proteins and DNA; the data also demonstrate a significant increase of the concentration of the histone proteins bound to DNA by electrostatic interactions, which can be overcomed by chloroform-isoamylic alcohol deproteinization in both low and high ionic strength medium in the absence or in the presence 1 M $NaClO_4$. Such interactions become normal following biotin treatment.

All the results obtained show that biotin administration causes in the blood of old rats (28 months) an increase in ATP, glucose, triglycerides, alkaline phosphatase and a significant decrease in cholesterol and acid phosphatase; moreover the biotin administration increases in the old rat liver the amount of DNA and of the electrostatic interactions between DNA and histones: the values in the old rats treated with biotin come within the values shown by adult rats. This regulatory activity of biotin on the biochemical pathways is probably related to ATP-synthesis, which in old rats is decreased.

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The alcohol syndromes: The intrarecombigenic effect of acetaldehyde

P. V. Véghelyi and Magda Osztovics

First Department of Paediatrics, Semmelweis University Medical School, and Human Genetics Laboratory, Bókay J. u. 53, H-1083 Budapest (Hungary), 6 July 1977

Summary. Sister chromatid exchange was studied in lymphocyte and fibroblast cultures. Alcohol caused no disturbance under normal conditions but an acetaldehyde level above 40 μM inhibited cell multiplication and elevated SCE considerably. A high acetaldehyde level is thought to elicit the fetal alcohol syndrome, a view supported by clinical and experimental observations.

It has been known for centuries that alcoholism has toxic consequences in the abuser and even in the offspring. In addition to alcoholic liver injury and delayed development of the fetus of alcoholic mothers, the close to 450 cases reported since 1968 leave no doubt about the existence of a fetal alcohol syndrome. 2 questions arise in this connexion: is the alcohol itself responsible for the syndrome; and why is only a fraction of the babies of addicts affected? We investigated these questions first in normal human lymphocyte cultures and in the cultured lymphocytes of alcohol-intoxicated subjects. Sister chromatid exchange (SCE) was studied applying 5-bromodeoxuridine treatment and 33258 Hoechst and Giemsa staining (FPG procedure)[1].

1. Ethanol added to the cultures at a final concentration of 0.5%, about the human lethal dose, did not increase SCE in comparison with alcohol-free controls.

2. From 7 alcohol addicts under the acute influence of alcohol, with blood levels ranging from 0.2 to 0.4%, lymphocytes were obtained and cultured in a 1:4 mixture of their own serum and TC 199 medium. In the 72 h cultures, the mitotic index was low, most of the cells were in the first metaphase, but there was no increase in SCE over the background. In subjects with a blood alcohol level under 0.2% even the cell cycle was normal. This result agreed with those[2,3] showing that alcohol does not prevent reproduction. At the same time, it might perhaps explain the lagging intrauterine development of the offspring of alcohol addicts.

Ethyl alcohol in the organism is metabolized in 2 steps. In the first it is oxidized by the hepatic enzyme alcohol dehydrogenase into acetaldehyde. In the second step, this compound is degraded by the ubiquitous enzyme aldehyde dehydrogenase. In further experiments, we have therefore studied the effect of acetaldehyde on the cultured lym-

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