Z. Ernährungswiss. **18**, 184–190 (1979)

ISSN 0044-264 X

Institute of Nutrition, Budapest (Hungary)
(Director: Prof. Dr. R. Tarján)

Hepatic metabolism in ethanol-treated young rats

M. Antal, Ö. Gaál, A. Gergely, K. Nagy, and *J. Szépvölgyi*

With 5 tables

(Received February 26, 1979)

The number of pregnant women, lactating mothers and young people ingesting ethanol regularly is increasing year by year. Scarce data are only available on the effects of ethanol on physiologic functions in the developing organism either in animals or in human beings (7, 14, 27).

In the present series on investigations we studied the effect of ethanol on some metabolic processes of the developing organism in rats ingesting ethanol from delivery (via the breast milk) or from weanling.

Materials and methods

Dams of a Wistar-derived strain, bred in our institute since 1949 (OETI strain) were offered a 15% aqueous ethanol solution as drinking fluid immediately after delivery until weanling. Weanling was the 28th day after delivery. Their offsprings also received the same drinking-fluid from weanling (Group A). An other group of rats received the 15% aqueous ethanol solution only after weanling (Group B). Siblings of rats in group B received tap water and served as controls (Group C). Both the 15% aqueous ethanol and tap water were offered ad libitum. Animals were fed a normal rat's chow (LATI, 369 kcal per 100 g). The individual fluid and food consumption and body weights were measured at intervals.

A few days prior to sacrifice hematocrit was determined in the tail blood using an AB Lars-Ljunberg hematocrit centrifuge.

All of the animals were sacrificed by decapitation at the end of the 12th week. SGOT and SGPT activities were assayed by Galenopharm testkit. Livers were promptly removed and weighed. Parameters for hepatic metabolism were studied in the livers of 10–10 rats.

Hepatic glycogen content was estimated by the method of *Good* et al. (12) using o-toluidine reagent (4). Total protein content was determined by Kjeldahl's method.

Hepatic triglycerides were extracted by the technique of *Folch* et al. (8) and quantitated by Boehringer UV test.

Liver samples (1 g/10 ml) were homogenized in a Potter-Elvehjem type teflon glass-tube in 0.25 M sucrose medium. The homogenate was centrifuged at 10,000 × g for 15 min in a Beckman centrifuge (J-21B). The supernatant was spun at 105,000 × g for 60 min in an ultracentrifuge (Janetzki Vac 601). Glucose-6-phosphate dehydrogenase (G6P-DH) was assayed in the 105,000 × g supernatant by the method of *Glock* and *McLean* (10) as modified by *Löhr* and *Waller* (20), alcohol dehydrogenase (ADH) was assayed according to the method described by *Bücher* and *Redetzki* (6). In the G6P-DH or ADH activity assays the kinetics of the generation of NADPH or NADH was followed in a Unicam AC 30 Automatic chemistry system at 340 nm and 25 °C.

Glucose-6-phosphatase (G6P-ase) activity was determined in the 105,000 × g sediment by the method of *Baginski* (3).

Table 1. Body weight of rats subjected to different treatments.

Sex and group	Weeks after weanling												
	0^+	1	2	3	4	5	6	7	8	9	10	11	12
	Body weight (g)												
Male													
A	$37^{a'b'}$	$62^{a'b'}$	$81^{a'b'}$	$101^{a'b'}$	$131^{a'b'}$	$136^{a'b'}$	$195^{a'b'}$	$224^{a'b'}$	$239^{a'b'}$	$254^{a'b'}$	$265^{a'}$	$274^{a'}$	$288^{a'b'}$
B	73	$93^{c'}$	$114^{c'}$	$138^{c'}$	175	205	235^{c}	248^{c}	$262^{c'}$	285^{c}	292^{c}	$298^{c'}$	$317^{c'}$
C	71	112	143	166	188	221	255	291	300	306	315	333	354
Female													
A	$39^{a'b'}$	$61^{a'b'}$	$70^{a'b'}$	$90^{a'b'}$	$114^{a'b'}$	$130^{a'b'}$	$152^{a'b'}$	$168^{a'b'}$	$181^{a'b'}$	$192^{a'b'}$	$195^{a'b'}$	$202^{a'b'}$	$207^{a'b'}$
B	72	$86^{c'}$	$95^{c'}$	$112^{c'}$	$142^{c'}$	$162^{c'}$	$177^{c'}$	$191^{c'}$	$194^{c'}$	$210^{c'}$	$218^{c'}$	$222^{c'}$	230^{c}
C	71	105	128	156	180	191	203	222	226	234	241	244	251

Group A: consumed ethanol from birth; Group B: consumed ethanol from weanling; Group C: control group; 0^+: weanling (on 28th day after delivery).

Data are means. Values marked with superscripts differ significantly (Student's t test) from those compared:

Groups compared	Significance
a : A and C	$p < 0.05$
a' : A and C	$p < 0.01$
b : A and B	$p < 0.05$
b' : A and B	$p < 0.01$
c : B and C	$p < 0.05$
c' : B and C	$p < 0.01$

Table 2. Daily food and fluid consumptions of rats after weanling with different treatments.

Sex and group	Food intake g/100 g bw	Calorie intake by food kcal/100 g bw	Fluid intake ml/100 g bw	Ethanol intake g/100 g bw	Calorie intake by ethanol kcal/100 g bw
Male					
A	8.17 + 3.68[a]	30.16 ± 13.59[a]	13.59 ± 4.57[a']	1.63 ± 0.54	11.57 ± 3.83
B	8.02 ± 2.99[c]	29.59 ± 11.04[c]	12.08 ± 3.76[c']	1.45 ± 0.45	10.30 ± 3.20
C	10.08 ± 4.46	37.20 ± 16.48	19.29 ± 5.26	–	–
Female					
A	8.73 ± 3.07[a]	32.21 ± 11.35[a]	10.75 ± 2.45[a']	1.29 ± 0.29	9.16 ± 2.06
B	7.58 ± 3.02[c']	27.96 ± 11.10[c']	12.25 ± 2.03[c]	1.47 ± 0.24	10.43 ± 1.70
C	10.77 ± 3.64	39.75 ± 13.44	14.56 ± 4.72	–	–

Data are means ± SD.
For further details see legend to table 1.

To assay the activities of mixed function oxygenases liver samples were homogenized in 1.15% KCl in 1:5 ratio.

Aminopyrine demethylase (APDM) and aniline hydroxylase (AHO) activity were determined in the 10,000 × g supernatant using aminopyrine and aniline as substrates (16).

Protein contents of various fractions were determined by the method of *Lowry* et al. (21).

Enzymic activities are expressed in μmole · min^{-1} · mg^{-1} protein for G6P-DH, ADH and G6P-ase or μmole · h^{-1} · mg^{-1} protein for APDM and AHO.

Zn contents of the 105,000 × g supernatant were estimated in a Perkin-Elmer 403 atomic absorption spectrophotometer with acetylene-air flame.

For histological studies liver sections were stained with hematoxyline-eosine.

Statistical significance was calculated by Student's t test. Data are presented as means ± SD.

Results

The body weights of rats consuming ethanol already via the milk (Group A) were significantly lower than those of controls (Group C). The growth rate of rats (Group B) consuming ethanol from weanling were retarded as soon as the 1st week of study and their body weights remained significantly lower than controls (Group C) throughout the whole observation period (table 1).

The mean daily food consumption calculated for 100 g body weight for all of the ethanol-treated rats was lower than that for controls. On the other hand, the total calorie intake reached and even exceeded that of controls if we presume that 1 g ethanol is equivalent to 7.1 kcal (19).

In response to ethanol, fluid consumption was decreased in both female and male groups, the difference being more significant in males (table 2).

Despite the lower fluid consumption hematocrit values were almost identical (table 3).

Table 3. Hematocrit and serum enzyme activities of rats with different treatments.

Sex and group	Hematocrit %	SGOT I.U./l	SGPT I.U./l
Male			
A	49 ± 1.5	44.4 ± 9.2	17.9 ± 2.4
B	48 ± 1.2	46.0 ± 9.5	16.6 ± 3.0
C	48 ± 0.9	46.5 ± 7.8	20.9 ± 3.4
Female			
A	45 ± 1.8	44.6 ± 11.4	15.3 ± 2.7
B	45 ± 1.7	39.0 ± 9.3	16.7 ± 3.0
C	44 ± 0.7	45.2 ± 7.2	18.5 ± 1.9

SGOT: Serum glutamate-oxaloacetate transaminase; SGPT: Serum glutamate-pyruvate transaminase.
Data are means ± SD.
For further details see legend to table 1.

No changes were seen in relative liver weight, hepatic protein content and Zn-content of the 105,000 × g supernatant in any of the ethanol-treated groups.

Hepatic triglyceride content increased significantly in the females of group A. Hepatic glycogen content decreased significantly in all of the ethanol-treated groups as compared to control values (table 4).

G6P-ase and AHO activities were elevated significantly in all of the ethanol-treated groups (table 5).

Discussion

The results of this study show that regarding the growth rate it is of basic importance whether ethanol consumption started already via the breast milk (Group A) or only from weanling (Group B). Despite the almost identical food consumption by rats in groups A and B, the weight difference between the two groups due to the effect of ethanol during the

Table 4. Effect of ethanol on some liver constituents in rats.

Sex and group	Liver weight g/100 g bw	Protein mg/g liver	Triglyceride mg/g liver	Glycogen mg/g liver	Zn content µg/mg protein
Male					
A	3.34 ± 0.18	220 ± 9	11.97 ± 2.07	29.33 ± 6.26[a']	0.176 ± 0.028
B	3.16 ± 0.13	236 ± 12	12.00 ± 2.31	31.31 ± 3.51[c']	0.163 ± 0.066
C	3.33 ± 0.13	231 ± 18	13.24 ± 2.68	45.09 ± 11.73	0.181 ± 0.057
Female					
A	3.31 ± 0.16	234 ± 15	14.33 ± 2.87[a']	25.98 ± 5.28[a']	0.186 ± 0.031
B	3.27 ± 0.21	216 ± 23	11.85 ± 2.45	23.77 ± 4.34[c']	0.173 ± 0.017
C	3.48 ± 0.35	233 ± 24	10.26 ± 1.47	40.73 ± 11.70	0.186 ± 0.014

Data are means ± SD
For further details see legend to table 1.

Table 5. Effect of ethanol on some hepatic enzymes in rats.

Sex and group	ADH	G6P-DH	G6P-ase	APDM	AHO
Male					
A	0.022 ± 0.002	0.014 ± 0.002	0.282 ± 0.046[a']	0.076 ± 0.013	0.022 ± 0.004[a']
B	0.020 ± 0.005	0.015 ± 0.004	0.354 ± 0.011[c']	0.084 ± 0.011	0.022 ± 0.003[c']
C	0.021 ± 0.001	0.012 ± 0.002	0.198 ± 0.056	0.073 ± 0.011	0.013 ± 0.002
Female					
A	0.022 ± 0.003	0.037 ± 0.008	0.368 ± 0.090[a']	0.055 ± 0.007	0.015 ± 0.002[a']
B	0.020 ± 0.003	0.043 ± 0.006	0.355 ± 0.054[c']	0.055 ± 0.077	0.014 ± 0.002[c']
C	0.023 ± 0.002	0.038 ± 0.009	0.190 ± 0.051	0.049 ± 0.004	0.008 ± 0.001

ADH: Alcohol dehydrogenase; G6P-DH: Glucose-6-phosphate dehydrogenase; G6P-ase: Glucose-6-phosphatase; APDM: Aminopyrine demethylase; AHO: Aniline hydroxylase.
Enzyme activities are expressed in μmol per mg protein per min for ADH; G6P-DH and G6P-ase and in μmole per mg protein per hour for APDM and AHO.
Data are means ± SD.
For further details see legend to table 1.

breast-feeding was maintained throughout the whole observation period. Although, the food consumption by ethanol-treated rats was lower than that of the controls nevertheless the total calorie intake in all experimental groups reached or exceeded the respective value for controls.

The mean daily fluid consumption as calculated for 100 g b.w. was in all ethanol-treated groups significantly lower than that in the controls. This difference was more pronounced in male groups. But the growth rate of ethanol-treated male rats was not lower than that of their female counterparts. No changes were seen in hematocrit, relative liver weight, hepatic protein content in response to ethanol. No toxic effects or signs of dehydration were observed by light microscopy. Thus, the decreased fluid consumption cannot satisfactorily explain the decreased growth rate of ethanol-treated rats. *Tze* and *Lee* (32), *Lieber* et al. (17) and *Lieber* (18) under different experimental conditions have also described growth retardation in response to ethanol which they attributed to the direct action of ethanol.

No elevation in ADH activities were noted in either of the ethanol-treated groups. Since ADH is a Zn-containing enzyme, the Zn content of the 100,005 × g supernatant was also determined, but no changes were observed. Literature contains conflicting reports concerning the changes of ADH activity due to the effect of ethanol. In this respect *Goebell* and *Bode* (11) suggested that the activity of ADH depends essentially on the time of exposure to ethanol.

As concerns the enzymes involved in the elimination of foreign substances, the rate of demethylation of aminopyrine did not differ in the various groups. But the hydroxylation of aniline was augmented by ethanol irrespectively of sex or age at beginning administration. Elevation of AHO activity due to ethanol has also been reported (28, 29).

Hepatic glycogen level was diminished in all of the ethanol-treated groups. In our earlier studies (2) we could not show this effect in R/Amsterdam rats treated alike. *Ammon* and *Estler* (1) used a 15% aqueous ethanol solution as drinking-fluid and found no decrease in hepatic glycogen content even after 6 months. *Ramakrishnan* et al. (26) reported a diminution of hepatic glycogen synthesis in rats due to ethanol in a long-term study. In addition to the suppressed synthesis, the increased glycogen breakdown is also feasible as indicated by the elevated activity of microsomal G6P-ase. An augmentation of G6P-ase activity in response to ethanol has also been reported (15, 24, 25) in acute and subacute studies.

Hepatic triglyceride content was somewhat higher in male control rats than in females. Similar sex difference was observed by *Bedö* in OETI rats, other authors (31) could show such a difference only under certain feeding conditions. In our studies hepatic triglyceride content was increased significantly in female rats consuming ethanol already via the breast-milk. *Mallov* and *Bloch* (22) have observed a more pronounced elevation of hepatic lipid content in female than in male rats under the effect of acute ethanol treatment. Acute studies performed in OETI rats resulted in similar findings. It appears that the responses of female OETI rats are more intense in both acute or subacute ethanol studies. On the other hand, using the same experimental protocol in R/Amsterdam rats hepatic triglyceride content remained unaffected in ethanol treated groups (2).

Morgan et al. (23) reported on the elevation of hepatic lipid content due to the effect of ethanol in a subacute study, whereas *Scheig* et al. (30) failed to demonstrate the augmentation of hepatic triglyceride content under the effect of a long-term ethanol treatment in rats. *Furuno* et al. (9) have recently shown that genetic factors play an essential role in the development of ethanol-induced fatty liver.

Earlier studies (5) from this laboratory have shown that the physically active, lean R/Amsterdam rats preferably use lipids as fuel in response to physical load, whereas the less active, obese Wistar-derived OETI rats rather use carbohydrates under similar conditions. These metabolic differences might account for the decrease of hepatic glycogen content in OETI rats and for the increase of hepatic triglyceride level in the female group A in response to ethanol, while no similar changes took place in the livers of R/Amsterdam rats.

Both literature data and our present results suggest that the effects of ethanol essentially depend on the strain, age, sex of rats, and on the time of exposure.

Summary

Dams of a Wistar-derived OETI strain were offered a 15% aqueous ethanol solution as drinking-fluid immediately after delivery until weanling. Their offsprings also received the same drinking-fluid from weanling (Group A). Another group of rats received the 15% aqueous ethanol solution only after weanling (Group B). In response to ethanol treatment the retardation of growth was the most marked in Group A, but the growth of rats in Group B was also decreased significantly as compared to that of controls.

Hepatic glycogen content was diminished in all of the ethanol-treated groups. Triglyceride content was increased in the livers of female rats in Group A.

By comparing earlier results with the present findings the authors point to the importance of strain and sex in experiments when parameters for hepatic metabolism are studied in response to ethanol.

Zusammenfassung

Die OÉTI-Ratten vom Stamm Wistar haben Wasser mit einem Alkoholgehalt von 15% entweder von der Geburt an, schon mit der Muttermilch (Gruppe A) oder von der Milchentwöhnung an (Gruppe B) verbraucht. Der Zuwachs der Ratten der Gruppe A wurde durch den Alkohol am entschiedensten gehemmt sowie das Wachstum der Gruppe B, welches aber sehr bedeutend hinter dem der Kontrollgruppe zurückgeblieben ist.

Der Alkohol hat in allen Gruppen den Glykogengehalt der Leber verringert und hat den Triglyzeringehalt der Leber der zur Gruppe A gehörenden Weibchenratten erhöht.

Die Ergebnisse ihrer früheren Untersuchungen mit den Ergebnissen gegenwärtiger Untersuchungen vergleichend, betonen die Verfasser die Bedeutung des geprüften Stammes und des Geschlechts dann, wenn sie die Wirkung des Alkohols auf die einzelnen Parameter des Leberstoffwechsels untersuchen.

References

1. *Ammon, H. P. T., C. J. Estler:* Nature, **216,** 158 (1967). – 2. *Antal, M., Ö. Gaál, K. Nagy, A. Blaskovits, A. Gergely:* Acta Physiol. Hung. (in press). – 3. *Baginski, E. S., P. P. Foá, B. Zak:* Methods of enzymatic analysis (edited by *H. U. Bergmeyer*), 876–880 (Weinheim, New York, London 1974). – 4. *Bakos, Gy:* Orvosi Hetilap **107,** 2320 (1966). – 5. *Bedő, M., M. Krámer, R. Tarján,* Acta Physiol. Hung. **51,** 87 (1978). – 6. *Bücher, T., H. Redetzki:* Klin. Wschr. **29,** 615 (1951). – 7. *Czeizel, E., I. Palkovich:* Egészségtudomány **9,** 263 (1965). – 8. *Folch, J., M. Lees, S. G. H. Stanley:* J. Biol. Chem. **226,** 497 (1957). – 9. *Furuno, K., M. Arakawa, A. Shino, Z. Suzuoki:* J. Nutr. **105,** 1253 (1975). – 10. *Glock, G. E., P. McLean:* Biochem. J. **55,** 400 (1953). – 11. *Goebell, H., Ch. Bode:* Metabolic changes induced by alcohol (edited by *G. A. Martin* and *Ch. Bode*), 23–30 (Berlin–Heidelberg–New York 1971). – 12. *Good, C. A., H. Kramer, M. Somogyi:* J. Biol. Chem. **100,** 485 (1933). – 13. *Hill, P.:* Lipids **5,** 621 (1970). – 14. *Hollstedt, C., O. Olsson, U. Rydeberg:* Medical Biology **55,** 1 (1977). – 15. *Ishii, H., J. G. Joly, C. S. Lieber:* Metabolism **22,** 799 (1973). – 16. *Kato, R., J. R. Gillette:* J. Pharmacol. Exp. Ther. **150,** 279 (1965). – 17. *Lieber, C. S., D. P. Jones, L. M. De Carli:* J. Clin. Invest. **44,** 1009 (1965). – 18. *Lieber, C. S.:* New Engl. J. Med. **288,** 356 (1973). – 19. *Lieber, C. S.:* Sci. Amer. **234,** 25 (1976). – 20. *Löhr, G. W., H. D. Waller:* Methods of enzymatic analysis (edited by *H. U. Bergmeyer*) 636–643 (Weinheim, New York, London 1974). – 21. *Lowry, O. H., N. J. Rosebrough, A. L. Farr, R. J. Randall:* J. Biol. Chem. **193,** 265 (1951). – 22. *Mallov, S., J. L. Bloch:* Amer. J. Physiol. **184,** 29 (1956). – 23. *Morgan, A. F., L. Brinner, C. B. Plaa, M. M. Stone:* Amer. J. Physiol. **189,** 290 (1957). – 24. *Nelson, P., W. C. Tan, S. R. Wagle, J. Ashmore:* Biochem. Pharmacol. **16,** 1813 (1967). – 25. *Nelson, P., S. R. Wagle, J. Ashmore:* Metabolism **19,** 349 (1970). – 26. *Ramakrishnan, S., C. V. Prasanna, A. Balasubramanian:* Indian J. Biochem. Biophys. **11,** 321 (1974). – 27. *Rawat, A. K.:* Biochem. J. **160,** 653 (1976). – 28. *Rubin, E., F. Hutterer, C. S. Lieber:* Science **159,** 1469 (1968). – 29. *Rubin, E., P. Bacchin, H. Gang, C. S. Lieber:* Lab. Invest. **22,** 569 (1970). – 30. *Scheig, R., N. M. Alexander, G. Klatskin:* J. Lipid Res. **7,** 188 (1966). – 31. *Sugawa-Katayama, Y., N. Morita:* J. Nutr. **105,** 1377 (1975). – 32. *Tze, W. J., M. Lee:* Nature **275,** 479 (1975).

Authors' address:

Dr. *M. Antal,* Institute of Nutrition, Budapest, Gyáli út 3/a. H-1097 Budapest