

Table 1. PERFORMANCE OF RATS IN TILTED PLANE TEST DURING ETHANOL INTOXICATION

Exp. series	Number of animals		Age of animals, weeks		Performance in test		Sexes different, $P <$	Blood alcohol (mgm.%)	
	M	F	M	F	M	F		M	F
I	10	15	20	14	66.9±4.8	74.7±6.1	0.005	202±11	207±16
II	10	10	15	11	68.7±5.9	68.7±5.3	NS	202±11	196±14
III	14	14	14	14	68.8±6.0	75.7±5.1	0.001	210±16	206±14
	14	14	18	18	64.2±5.0	69.3±4.4	0.01		
	14	14	22	22	62.0±1.9	63.2±4.1	NS		

The results expressed as per cent of an initial 'sober' value obtained immediately before alcohol injection. The lowest value observed in 6 tests during 90 min. following injection is given. Standard deviation is indicated. In series III, the same individuals were tested at three different ages. The means from all 6 tests in one experimental run gave comparable results.

from the tail, immediately after the final testing, for analysis according to the method of Newman and Newman², modified to allow the use of approximately 100 mgm. of blood. The results are shown in Table 1. The performance of the animals indicates that the tolerance of females increases transiently when breeding maturity is reached. It returns to the same level as that of the males in about 8 weeks.

The higher tolerance of the females is not due to differences in rate of alcohol oxidation, since no significant difference in blood alcohol level was found. Ijiri³ observed that a 1 per cent alcohol solution increased *in vitro* oxygen consumption of unstimulated cerebral cortex and mid-brain tissue from normal rats, whereas even a 0.5 per cent solution depressed the oxygen consumption of corresponding tissues from castrated animals. Goldberg and Störtebecker⁴ have reported an anti-narcotic effect of oestrone on alcohol intoxication in castrated female rabbits and conclude that the resistance of the central nervous system is related to the hormonal state. Angelucci⁵ has demonstrated a sex difference in rats with respect to morphine tolerance, females being more resistant than males. Female rats tolerate chlorpromazine better than do male rats, and the tolerance of males is reduced with advancing age.⁶

The present observation has obvious relevance for the selection of animals for experiments on the effects of alcohol. Whether a change in the general response to stressors or some specifically nervous mechanism is involved cannot be judged on basis of this material.

HENRIK WALLGREN

Research Laboratories, State Alcohol Monopoly,
Helsinki, Finland. June 3.

¹ Arvola, A., Sammalisto, L. and Wallgren, H., *Quart. J. Stud. Alc.*, **19**, 563 (1958).

² Newman, E. J. and Newman, H. W., *Stanford Med. Bull.*, **11**, 98 (1953).

³ Ijiri, J., *Mitt. med. Akad. Kioto*, **26**, 813 (1939).

⁴ Goldberg, L. and Störtebecker, T. P., *Acta Physiol. Scand.*, **5**, 289 (1943).

⁵ Angelucci, L., *Nature*, **181**, 967 (1958).

⁶ Hoffman, R. A. and Zarrow, M. X., *Amer. J. Physiol.*, **193**, 547 (1958).

α -Ketoglutaric Acid and Pyruvic Acid in Blood, Cerebrospinal Fluid and Urine

DETERMINATIONS of α -ketoglutaric acid and pyruvic acid in blood, cerebrospinal fluid and urine have been carried out using 2, 4-dinitrophenylhydrazine method.¹ The keto-acid hydrazones were separated, either by paper electrophoresis or by paper chromatography.

The electrophoretic separation² was carried out in 0.05 M sodium bicarbonate at 400–420 V./10–18 m.amp. for 3 hr. on Whatman No. 1 paper (20 × 29 cm.). The chromatographic separation³ was per-

formed in *n*-butanol-ethanol-1 per cent ammonia mixture (6 : 1 : 3 v/v). The amount of hydrazones applied at the start corresponded to 0.5 ml. of blood or urine respectively, or to 1 ml. of cerebrospinal fluid. After separation the hydrazone spots (both isomers in the case of pyruvic acid) were extracted with 1 N sodium carbonate and measured at 380 m μ on the Zeiss spectrophotometer.

Higher values of pyruvic acid in electrophoretic separation (Table 1) are due to the fact that together with pyruvic acid other α -keto-acids (eventually aldehydic acids) found in traces only in the biological material, are determined and their hydrazones travel in the electric field with the same speed as hydrazone of pyruvic acid does. As it was formerly shown in the case of pyruvic acid hydrazone, approximately the same mobility was observed for hydrazone of glyoxylic acid and phenylpyruvic acid (two isomers again), and for α -ketoisocaproic acid by Biserte and Dassonville.⁴ Both hydrazones mentioned above can be separated by chromatography.

Table 1. VALUES OF α -KETOGLUTARIC ACID AND PYRUVIC ACID IN BLOOD AND CEREBROSPINAL FLUID AS DETERMINED BY ELECTROPHORETIC AND CHROMATOGRAPHIC METHODS

	The number of cases	Chromatographically mgm./100 ml.		Electrophoretically mgm./100 ml.	
		α -keto-glutaric acid	pyruvic acid	α -keto-glutaric acid	pyruvic acid
Blood	12	0.15±0.07	0.41±0.11	0.14±0.08	0.50±0.14
Cerebrospinal fluid	6	not exceeding 0.04	0.48±0.12	not exceeding 0.04	0.54±0.14

In urine of 10 patients confined to bed and suffering from no metabolic disease 14.13±3.20 mgm. of α -ketoglutaric acid and 8.16±1.55 mgm. of pyruvic acid were found on average during 24 hr. Five employees of this institute carrying out their normal duties excreted 18.40±4.05 mgm. of α -ketoglutaric acid and 11.06±4.84 mgm. of pyruvic acid in 24 hr. Both physical and mental strain increase the amount of α -keto-acids eliminated in the urine.

Patients confined to bed excreted maximum values of keto-acids during the afternoon or evening hours. Women eliminated more α -keto-glutaric acid during the night than men.⁵

E. ZELNICEK

Department of Medical Chemistry,
Masaryk University,
Brno, Czechoslovakia. May 3.

¹ Zelnicek, E., *Scripta medica fac. med. univ. Brunensis et Olomucensis*, **30**, 291 (1957).

² Zelnicek, E., and Cernoch, K., *Cas. lek. ces.*, **97**, 188 (1958).

³ El Hawary, M. S. F., and Thompson, R. H. S., *Biochem. J.*, **53**, 340 (1953).

⁴ Biserte, G., and Dassonville, B., *Clin. Chim. Acta*, **1**, 49 (1956).

⁵ McArdle, B., *Biochem. J.*, **66**, 144 (1957).