TABLE 1. EFFECT OF TETRAETHYLAMMONIUM ION ON THE AFFINITY (K_a) AND THE PHOSPHORYLATION OR CARBAMYLATION CONSTANTS (k_2) FOR THE INHIBITION OF ACETYLCHOLINESTERASE WITH TETRAM, MALAOXON AND TEMIK

	Tetram alone	Tetram plus TEA	Malaoxon alone	Malaoxon plus TEA	Temik alone	Temik plus TEA
K_a (mM)	0·18 ± 0·01	0·46 ± 0·05	2·4 ± 0·15	7·8 ± 1·9	5·5 ± 0·6	11·0 ± 1·8
k_2 (min^{-1})	126 ± 6·4	8·0 ± 0·8	67·0 ± 2·6	8·3 ± 1·8	24·0 ± 2·6	17·4 ± 2·8
k_i (M^{-1} min^{-1})	7·1 × 10^5	0·17 × 10^5	28·0 × 10^3	1·1 × 10^3	4·3 × 10^3	1·6 × 10^3

In the presence of TEA, both the affinity and k_2 decreased, resulting in a 25-, 42- and 2·7-fold decrease in the overall inhibitory power (k_i) of malaoxon, Tetram and Temik respectively. The effect of TEA on the phosphorylation rate was of a much greater magnitude than its effect on the affinity. The k_2 of malaoxon in the presence of TEA showed an 8-fold decrease and the affinity was decreased 3·3-fold. The TEA ion affected the phosphorylation rate of Tetram even more than that of malaoxon. The k_2 decreased 16-fold and the k_i diminished about 42-fold. The affinity and carbamylation constants of Temik were affected only slightly. The k_2 was only 1·4-fold lower, while the affinity decreased 2-fold.

The data indicated that the presence of TEA significantly modified both the binding and phosphorylation or carbamylation constants of the three compounds studied. The findings did not clarify whether the α-carbonyl carbon bound at the anionic site, even though the affinity of malaoxon could be altered with TEA ion.

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REFERENCES

1. Y. C. CHIU and W. C. DAUTERMAN, *Biochem. Pharmac.* **18**, 359 (1969).
2. Y. C. CHIU and W. C. DAUTERMAN, *Biochem. Pharmac.* **18**, 1665 (1969).
3. W. B. NEELY, *Molec. Pharmac.* **1**, 137 (1965).
4. F. BERGMANN and A. SHIMONI, *Biochim. biophys. Acta* **8**, 520 (1952).
5. F. BERGMANN and A. SHIMONI, *Biochim. biophys. Acta* **10**, 49 (1953).
6. A. R. MAIN and F. IVERSON, *Biochem. J.* **100**, 525 (1966).

Biochemical Pharmacology, Vol. 19, pp. 1857–1859. Pergamon Press. 1970. Printed in Great Britain

Hepatic microsomal epoxidase in the cotton rat—Effect of dietary variables

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THE FUNCTIONAL integrity of hepatic microsomal membranes is known to be vital to the drug-metabolizing activities associated with this subcellular fraction.[1] Torula yeast diets can be used to produce a number of the symptoms of deficiency diseases, all having the common characteristic of a breakdown in cellular membrane structure and function. Some of these symptoms can be alleviated with vitamin

E, selenium, Schwarz Factor 3, or sulfur aminoacids,[2] or intensified by salts[3] or other vitamin insufficiencies. In this experiment with cotton rats (*Sigmondon hispidus texianus*), a comparison was made of the effects of the torula yeast diet and various supplements on a known deficiency symptom, cardiac calcification, and the activity of the liver microsomes in regard to their ability to epoxidize the insecticide, aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-endo-exo-dimethanonaphthalene). The hypothesis that cardiac calcification and microsomal epoxidase would respond similarly to dietary additions seems to be most unlikely, since they were affected to different extents and showed no significant correlation.

Male cotton rats (five animals per treatment) were placed on either a basal[4] or supplemented torula yeast diet (Table 1) approximately 3 weeks postpartum. They were kept in individual cages with food

TABLE 1. SPECIFIC ACTIVITIES OF EPOXIDASE FOR RATS ON BASAL AND SUPPLEMENTED TORULA YEAST DIETS, AND LAB CHOWS

Supplements to basal 201 diet kg of ration	No. of animals	Epoxidase activity (pmoles dieldrin prod./ mg Ms protein/min)	Calcification index
None	5	157 ± 58	1·2
Inositol (1 g)	4	191 ± 38	1·0
D,L-Methionine (5 g)	2	170 ± 54	2·5
K_2SO_4 (9 g)*	3	148 ± 7	0·7
Na_2SO_4 (7·5 g)*	1	115	0·0
K_2SO_4 (9 g)	3	149 ± 68	2·3
$CaSO_4$ (7·1 g)	2	171 ± 6	1·0
$MgSO_4$ (6·3 g)	3	146 ± 7	0·3
d-α-tocopherol acetate (60 mg) + K_2SO_4 (9 g)	5	295 ± 17†	2·2
Sodium selenite (0·1 mg) + K_2SO_4 (9 g)	4	236 ± 35‡	2·8
Linseed oil (5% in place of lard) + K_2SO_4 (9 g)	2	298 ± 62‡	1·0
Linseed oil (5% in place of lard)	4	323 ± 60†	0·3
Ethyl ether-extracted torula yeast + K_2SO_4 (9 g)§	3	247 ± 57‡	1·0
Ethyl ether-extracted torula yeast§	3	307 ± 5†	2·3
Purina lab chow	4	311 ± 109‡	0·0

* B_{12} injections given subcutaneously: 10 μg on day zero and 12·5 μg on day 35.
† Statistically significant with respect to basal 201 diet at the 1% level.
‡ Statistically significant with respect to basal 201 diet at the 5% level.
§ Basal torula yeast diet was made with torula yeast which had been extracted with ethyl ether first.

and water *ad lib*. After 8 weeks on the diet, the animals were sacrificed by CO_2 inhalation and the hearts and liver were removed. The hearts were histologically examined for calcification by staining with hematoxyline and eosin. Calcification was rated on an index of zero to five. The microsomes were isolated from the livers by differential centrifugation.[5] The aldrin epoxidase activity was determined the morning after the isolation of the microsomes.[5] Four rats, 10 weeks old, raised on Purina lab chows were also sacrificed and examined for epoxidase activity and heart calcification. The procedure of Lowry *et al.*[6] was used for microsomal protein determinations with bovine serum albumin as a standard.

The specific enzyme activity is expressed as (picomoles of dieldrin produced) (mg microsomal protein)$^{-1}$(min)$^{-1}$. The averages are reported with an estimate of the population standard deviation. Population means were compared by Student's *t*-test.[7]

The basal and supplemented torula yeast diets do not affect body growth patterns. The details of the rat's development and heart calcifications will be discussed in another paper.* The ratio of liver weight to total body weight and the milligrams of microsomal protein isolated per gram of liver homogenized are similar in all cases. Table 1 summarizes the specific activity of epoxidase.

An attempt was made to correlate the epoxidase activity with the heart calcification index for each animal. The small correlation coefficient ($R = -0{\cdot}005$) indicates that a correlation is highly improbable. Although no relationship was found, the epoxidase activities do fall into two groups worth

* H. Pendell, in preparation.

noting. The rats raised on the diet supplemented with vitamin E, selenium, linseed oil and the diet compounded with ethyl ether-extracted torula yeast had epoxidase activities comparable to those of rats fed the lab chows and 2-fold greater than those of rats raised on the basal diet or on the diet supplemented with inositol, methionine or various salts.

Work on other hepatic microsomal enzyme systems shows that the dietary level of vitamin E can influence enzyme activity.[8-10] Vitamin E may be involved directly in membrane structure and stability,[11] but could also be acting as a cofactor in enzyme activity. Selenium often alleviates the same deficiency diseases as does vitamin E, possibly through a general antioxidant role. Linseed oil, high in polyunsaturated fatty acids, could be protecting or supplementing peroxidizable endogenous fatty acids involved with some membrane activity. The possibility of a supressant in the torula yeast, compensated for by vitamin E, selenium or linseed oil, and removed by ethyl ether extraction, is currently under investigation. It is unknown whether or not these dietary components, which increase the enzyme activity, operate under a general mechanism or have separate specific functions.

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REFERENCES

1. L. ERNSTER, P. SIEKEVITZ and G. E. PALADE, *J. Cell Biol.* **15**, 541 (1962).
2. K. SCHWARZ, *Nutr. Rev.* **18**, 193 (1960).
3. M. A. CONSTANT and P. H. PHILLIPS, *J. Nutr.* **47**, 317 (1952).
4. J. F. BONE, S. A. ROFFLER and P. H. WESWIG, *Lab. Anim. Care* **15**, 81 (1965).
5. J. W. GILLETT, T. M. CHAN and L. C. TERRIERE, *J. agric. Fd Chem.* **14**, 540 (1966).
6. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951),
7. W. MENDENHALL, *Introduction to Probability and Statistics*, pp. 41, 189-201. Wadsworth, Belmont. Calif. (1967).
8. M. P. CARPENTER, *Fedn Proc.* **26**, 475 (1967).
9. M. P. CARPENTER, *Fedn Proc.* **27**, 677 (1968).
10. T. E. GRAM and J. R. FOUTS, *Archs Biochem. Biophys.* **114**, 331 (1966).
11. O. R. ANDERSON, O. A. ROELS, K. D. DREHER and J. H. SCHULMAN, *J. Ultrastruct. Res.* **19**, 600 (1967).