Path. and Bact., 1936, v42, 1.

2. Steinberg, B., Martin, R. A., *Arch. Path.*, 1946, v41, 1.

3. Seller, E. A., Lucas, C. L., Best, C. H., *Brit. Med. J.*, 1948, v1, 1061.

4. Gyorgi, P., Goldblatt, H., *J. Exp. Med.*, 1949, v90, 73.

5. Jaffe, E. R., Wissler, R. W., and Benditt, E. P., *Am. J. Path.*, 1950, v26, 951.

6. Morrione, Th. G., *Am. J. Path.*, 1949, v25, 273.

Received December 9, 1952. P.S.E.B.M., 1953, v82.

Competitive Inhibitive Action of Normal Fatty Acids on Mammalian D-Amino Acid Oxidase. (20014)

W. T. BROWN AND P. G. SCHOLEFIELD. (Introduced by J. H. Quastel.)

From the Research Institute, Montreal General Hospital, Montreal, Canada.

During an investigation of the effect of various carboxylic compounds on the metabolism of mammalian tissues it was found that some of the simple normal fatty acids are competitive inhibitors of the mammalian D-amino acid oxidase. This action of the simple fatty acids does not seem to have been reported although Klein and Kamin(1) noticed an inhibition of D-amino acid oxidase by sodium benzoate. Later Bartlett(2) showed that the inhibition was competitive and that of many derivatives tested *m*-methyl benzoate was most effective. Zeller and Maritz(3) found that benzoate also inhibits ophio-L-amino acid oxidase and that other aromatic carboxylic and sulphonic acids had similar effects. The short chain normal fatty acids are shown to be more specific in their action; they inhibit the mammalian D-amino acid oxidase but have no action on ophio-L-amino acid oxidase.

Materials and methods. The source of the mammalian enzyme was a rat kidney homogenate. The animal was decapitated, exsanguinated, the kidneys removed and chilled on cracked ice. They were defatted, cut into small pieces and homogenized in 7.0 ml ice cold saline for 2 minutes. The homogenate was filtered through two layers of cheesecloth and 1.0 ml used per vessel. The substrate in all experiments reported was DL-methionine. The results were confirmed in most cases with DL-alanine as substrate, and it was shown that L-alanine was not oxidised by the rat kidney homogenate. The activity of the amino acid oxidase was followed in the Warburg apparatus by measuring oxygen uptake at 37°C with KOH filter paper in the centre well. In general, constant rates of oxygen uptake were found for 20 minutes and the inhibitions reported are inhibitions of this initial constant rate of oxygen uptake. Dried cobra venom was obtained from Hynson, Westcott and Dunning Ltd. (Baltimore) and 3.0 mg was used per vessel as the source of the ophio-L-amino acid oxidase.

Results. All the normal fatty acids from acetic to octanoic were tested as inhibitors of

TABLE I. Oxidation of DL-methionine by Rat Kidney Homogenate in Presence of the Sodium Salts of the Short Chain Fatty Acids.

Additions*	μl oxygen taken up in 20 min.	% inhibition
Nil	23	0
Methionine	146	
Sodium acetate	18	0
" " + methionine	145	
" propionate	18	0
" " + methionine	141	
" butyrate	24	39
" " + methionine	100	
" pentanoate	18	69
" " + methionine	56	
" hexanoate	15	50
" " + methionine	76	
" heptanoate	16	57
" " + methionine	69	
" octanoate	14	7
" " + methionine	128	

* All vessels contained 1 ml rat kidney homogenate, 1 ml .10 *M* phosphate buffer pH 7.4 and water to make a final vol of 3 ml. All the sodium salts were present at a final concentration of .01 *M* and the methionine at .02 *M*.

rat kidney D-amino acid oxidase; the results are shown in Table I. Sodium pentanoate was the most effective inhibitor and both the highest and lowest chain length fatty acids tested were ineffective. The nature of the inhibition by the sodium pentanoate was investigated using varying quantities of substrate and inhibitor. The results, plotted by the method of Lineweaver and Burk(4), are shown in Fig. 1. The inhibition is obviously competitive, the K_m for D-methionine in this system being approximately 8 x 10^{-3} *M* and the molar ratio of D-methionine to sodium pentanoate for 50% inhibition is of the order of 7.5:1.

In contrast to its effect on D-amino acid oxidase, sodium pentanoate failed to inhibit the ophio-L-amino acid oxidase of dried cobra venom even when the molar ratio of DL-methionine to pentanoate was 1:1. (Table II). The observed effects are therefore more specific than those of benzoate which inhibits both the kidney and the venom enzymes.

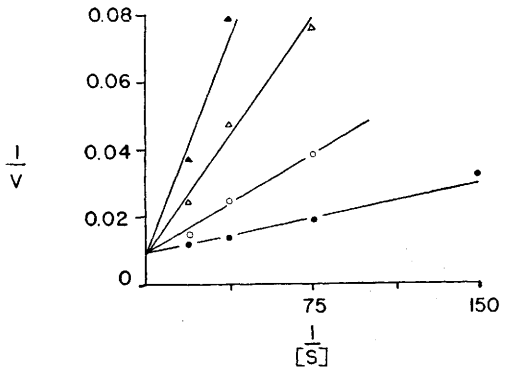

FIG. 1. Competitive inhibition of DL-methionine oxidation by sodium pentanoate. The velocity (V) is expressed as μl oxygen taken up in 12 min. (corrected for the endogenous respiration) and the DL-methionine concentration [S] is expressed as molarity. The reciprocals of these values are plotted. ● No sodium pentanoate present, ○ 3.3 × 10^{-3} *M*, △ 1 × 10^{-2} *M*, ▲ 2 × 10^{-2} *M* sodium pentanoate present. Other conditions as in Table I.

TABLE II. Oxidation of DL-methionine by Cobra Venom in Presence of Sodium Pentanoate.

Additions*	μl oxygen taken up in 60 min.
Nil	11
Methionine	167
Sodium pentanoate	12
" " + methionine	161

* All vessels contained 3 mg crystalline cobra venom, 1 ml .10 *M* phosphate buffer pH 7.4 and water to make a final vol of 3 ml. The final concentration of both methionine and sodium pentanoate was .02 *M*.

Summary. Some of the short chain normal fatty acids are shown to be competitive inhibitors of the D-amino acid oxidase of rat kidney. In contrast to benzoate, which is a competitive inhibitor of this enzyme and of the ophio-L-amino acid oxidase of cobra venom, sodium pentanoate inhibits only the kidney enzyme.

Grateful acknowledgement is made to the National Cancer Institute of Canada for a grant in aid of a programme of work of which the above represents a part, and to Dr. J. H. Quastel of this Institute for his continued interest in this work.

1. Klein, J. R., and Kamin, H., *J. Biol. Chem.*, 1941, v138, 507.

2. Bartlett, G. R., *J. Am. Chem. Soc.*, 1948, v70, 1010.

3. Zeller, E. A., and Maritz, A., *Helv. Chim. Acta.*, 1945, v28, 365.

4. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, 1934, v56, 658.

Received December 11, 1952. P.S.E.B.M., 1953, v82.