Table 2. R_F VALUES OF SYNTHETIC, ELUTED AND MIXED DINITROPHENYL γ-AMINO-BUTYRIC ACID

Solvent	Synthetic	Eluted	Mixed
(1) *n*-Butanol saturated with ammonia	0·40	0·40	0·39
(2) *n*-Butanol : water : ethanol (4 : 2 : 1)	0·90	0·89	0·90
(3) *n*-Butanol : acetic acid : water (4 : 1 : 5)	0·95	0·95	0·95
(4) Pyridine : water (3 : 2)	0·82	0·81	0·81

chromatography, by Koch and Weidel's method[6]. The choroid, iris and ciliary body of dog and ox were examined in the same way.

The dinitrophenyl γ-amino-butyric acid, eluted from paper, was identical with a synthesized sample by one-dimensional paper chromatography, exhibiting a single spot even in mixed chromatography (Table 2).

γ-Amino-butyric acid was found in the retina and choroid, but not in the iris and cilliary body. The possibility that it may be produced in the former tissues by the decarboxylation of glutamic acid is being investigated.

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Effect of Calcium Ions on the Inhibition of Hydrolases by Chlortetracycline

CHLORTETRACYCLINE is an inhibitor of some enzymatic reactions[1–5]. So far as the inhibition of hydrolases is concerned, reports in the literature are contradictory[6–9]. We demonstrated in previous work that pancreatic and mould α-amylase[10,11] and pancreatic lipase are inhibited by aureomycin (Lederle). It was possible to prevent the inhibitive effect of this antibiotic substance by adding citrate, oxalate and the sodium salts of other organic acids. If, however, pure chlortetracycline was used inhibition did not occur. It was assumed that citrate prevented the inhibitive effect by removing calcium, which is present in the usual clinical Lederle preparation or in raw enzymatic preparations, from the system.

For these reasons, we studied the influence of different concentrations of calcium on the effect of chlortetracycline during enzymatic hydrolysis of starch and tributyrin. The pancreatic juice from dogs and an aqueous extract of fat-free dried hog pancreas were used as sources of lipase and α-amylase.

Dog pancreatic juice was diluted (1 : 1,000) in such a way that the amount of calcium added with the enzymatic preparation was negligible. Pure chlortetracycline containing neither calcium nor other bivalent cations was used. The enzyme was incubated with tributyrin in an acetate buffer *p*H 6·0, final concentration 0·13 *M*, and agitated for 1 hr. at 28° C.

It was found that calcium (added to the system as chloride) influences the effect of chlortetracycline during hydrolysis of tributyrin by canine pancreatic

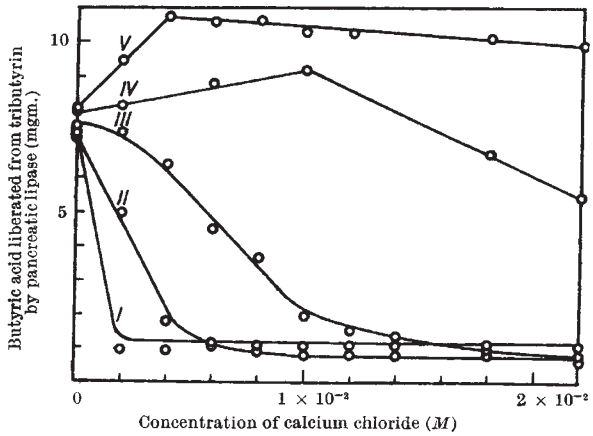

Fig. 1. I, 160 μgm.; II, 80 μgm.; III, 50 μgm.; IV, 40 μgm. chlortetracycline in 1 ml.; V, control

juice. Fig. 1 shows that the inhibitive effect of chlortetracycline increases with increasing concentrations of calcium. At a certain level of inhibition there is an indirect relation between the amount of calcium and that of chlortetracycline. In the absence of calcium or other bivalent cations chlortetracycline had no inhibitive effect. Similar results were obtained for hydrolysis of starch by pancreatic α-amylase. In the latter case, however, inhibition occurs only at a concentration of 200 μgm. chlortetracycline/ml. if sufficient calcium is present, whereas in the case of lipase 50 μgm./ml. already has an inhibitive effect (Fig. 1). Manganese had a similar influence on the effect of chlortetracycline on the hydrolysis of tributyrin by pancreatic lipase. Magnesium had less effect.

Some contradictory reports in the literature concerning the effect of chlortetracycline on enzymes such as lipase and α-amylase can be explained on the basis of our results, since some preparations of chlortetracycline contain calcium salts, as do also the impure enzymatic systems used by various authors when studying this question. Our previous results, showing that sodium citrate prevented the inhibitive effect of aureomycin on lipase and α-amylase, can also be explained by the removal of calcium ions from the system or a decrease in their concentration.

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