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INHIBITION OF PANCREATIC LIPASE BY TETRACYCLINES*

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ABSTRACT

The effect of various antibiotics on the rate of hydrolysis of an olive oil emulsion by hog pancreatic lipase was studied by potentiometric titration. Tetracycline, demethylchlortetracycline and doxycycline were able to inhibit this lipase system completely, at appropriate drug concentrations. None of the other drugs tested were able to affect this enzyme system significantly. These included penicillin G, ampicillin, oxacillin, erythromycin, chloramphenicol, streptomycin, sulfasoxazole and sulfamethoxazole.

The improvement in acne which often accompanies the administration of tetracycline has been attributed to a reduction in free fatty acid formation within the pilosebaceous canal (1). Since *Corynebacterium acnes* appears to possess lipolytic activity (2), is sensitive to tetracycline *in vitro* (3), and is reduced on the skin surface during the administration of this antibiotic (4), it is logical to assume that this decrease in fatty acids is due to the bacteriostatic effect of the drug.

C. acnes, however, is also quite sensitive to penicillin (3), yet this antibiotic does not appear to have any effect on the titratable acidity of human sebum (5). This observation led Strauss and Pochi to postulate that other mechanisms might be involved in the effect of broad-spectrum antibiotics on free fatty acid formation in skin (5).

Rokos and co-workers have have previously described inhibition of pancreatic lipase by chlortetracycline (6). The present study was undertaken to verify this observation and to compare the action of several tetracyclines with various other antibiotics on this enzyme system.

MATERIALS AND METHODS

The rate of hydrolysis of an olive oil emulsion was determined by potentiometric titration. One unit of enzyme activity is equal to one micromole of acid produced per minute at 25° C under the specified conditions. The enzyme solution was prepared from purified hog pancreatic lipase (Worthington Biochemical Corporation 3.1.1.3). A final concentration of 1.0 mgm per ml was obtained by diluting the powdered enzyme in 0.005 M $CaCl_2$.

Substrate solution was prepared by pipetting the following reagents into a thermostated reaction vessel at 25° C over a magnetic stirrer:

2 ml Na Taurocholate (15 mgm per ml)
5 ml Olive Oil emulsion (obtained by blending 20 ml olive oil with 165 ml. 10% gum acacia and 15 gms crushed ice in a Waring Blendor for 10–15 minutes)
5 ml H_2O
2 ml NaCl (3.0 M)
1 ml $CaCl_2$ (0.075 M)

The pH of the substrate solution was adjusted to 8.1 by the addition of 0.04 N NaOH. A Heath, Malmstadt-Enke pH recording electrometer was used for all pH determinations. The reaction surface was continuously flooded with nitrogen during the titration.

After the addition of 0.25 ml. enzyme solution to the substrate, zero time was started when the reaction reached pH 8.0. The rate of base addition was determined for 5–6 minutes. Units of lipase activity were calculated according to the following formula:

$$\text{units/mgm. weight} = \frac{\text{ml NaOH/min.} \times \text{M NaOH} \times 1000}{\text{mgm. protein used}}$$

The effect of the different antibiotics was studied by adding varying concentrations of the purified, reference standard grade drug to the substrate solution immediately prior to the addition of enzyme. The pH was always adjusted to 8.1 before addition of the enzyme.

RESULTS

The results obtained with tetracycline, demethylchlortetracycline and doxycycline are outlined in Tables I–III. Percent inhibition by tetracycline is graphically represented in Figure 1.

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TABLE I

Percent inhibition of pancreatic lipase by tetracycline HCl. Control = 148.6 units*

Mgm. tetracycline HCl per ml substrate	Percent inhibition
0.0	0.0
0.062	0(−1.0)
0.125	3.1
0.187	9.0
0.250	40.8
0.312	47.2
0.375	74.2
0.430	91.4
0.500	96.7
0.562	98.0
0.625	100.0
0.750	100.0

* Supplied by Franklin M. Phillips, M.D., Lederle Laboratories, Pearl River, N.Y. and J. P. Jones, Jr., M.D., Pfizer Laboratories, New York, N.Y.

TABLE II

Percent inhibition of pancreatic lipase by demethylchlortetracycline. Control = 140 units*

Mgm. demethylchlortetracycline per ml substrate	Percent inhibition
0.0	0.0
0.312	0.0
0.500	0.0
0.625	57.1
0.750	71.0
0.937	100.0

* Supplied by Franklin M. Phillips, M.D., Lederle Laboratories, Pearl River, N.Y.

TABLE III

Percent inhibition of pancreatic lipase by doxycycline hyclate. Control = 146 units*

Mgm. doxycycline per ml. substrate	Percent inhibition
0.0	0.0
0.625	36.4
0.937	55.2
1.250	67.1
1.562	100.0

* Supplied by J. P. Jones, Jr., M.D., Pfizer Laboratories, New York, N.Y.

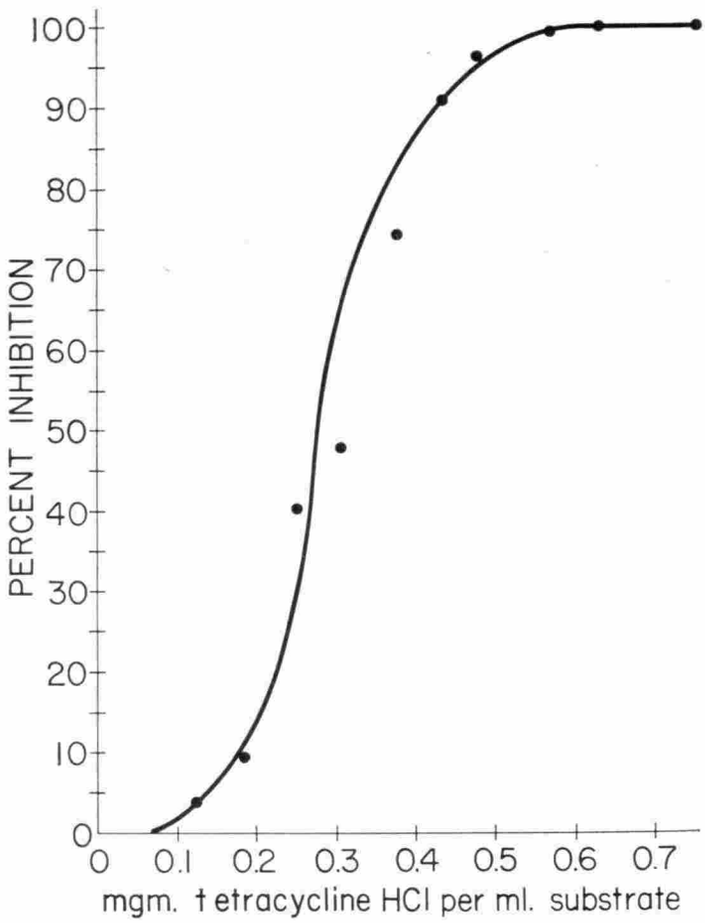

FIG. 1. Percent inhibition of lipase per mgm tetracycline.

These data indicate definite inhibition of hog pancreatic lipase by the tetracyclines in the system used. Maximum inhibition occurred with final concentrations of 0.625 mgm. per ml. tetracycline, 0.937 mgm. per ml. demethylchlortetracycline and 1.562 mgm. per ml. doxycycline. None of the other antibiotics tested had a statistically significant effect on this lipase system, even when used in concentrations up to ten times that of tetracycline. The other drugs tested were: potassium penicillin G,* sodium oxacillin,† ampicillin trihydrate,† streptomycin sulfate,* erythromycin stearate,‡ erythromycin lactobionate,‡ erythromycin ethyl succinate,‡ erythromycin lauryl sulfate,‡ erythromycin base,‡ erythromycin estolate,§ erythromycin glucoheptonate,§ chloramphenicol,¶ sulfasoxazole,‖ and sulfamethoxazole.‖

* Supplied by J. P. Jones, Jr., M.D., Pfizer Laboratories, New York, N.Y.

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‡ Supplied by Anne L. Barlow, M.D., Abbott Laboratories, North Chicago, Illinois.

§ Supplied by E. Linn Jones, M.D., The Lilly Research Laboratories, Indianapolis, Indiana.

¶ Supplied by H. E. Machaner, Ph.D., Parke Davis and Co., Detroit, Michigan.

‖ Supplied by W. E. Scott, Hoffman-La Roche, Inc., Nutley, N.J.

DISCUSSION

Previous workers have demonstrated inhibition of pancreatic lipase by chlortetracycline (6). They were, however, unable to determine any inhibition greater than 50 per cent. Our experiments indicate complete inhibition at appropriate antibiotic concentrations, and graphically, follow a typical inhibition curve. The difference between our results and those obtained by earlier workers could be the result of several differences in the experimental methods used. Our enzyme preparation is a relatively pure one, whereas earlier investigators appear to have employed a fairly crude extract. Furthermore, the addition of sodium taurocholate to our substrate provides a known enzyme activator which may affect the result. Also there could be a difference in reaction rate between the tributyrin used by Rokos *et al.* (6) and the olive oil emulsion used in these experiments which is primarily triolein. Finally, recent investigators have emphasized the importance of measuring the release of fatty acids by continuous titration (7), a procedure used by us, but not by the workers referred to above.

Of particular interest is the fact that among the antibiotics tested, only the tetracyclines appeared able to inhibit pancreatic lipase under these test conditions. While it is possible that this observation may help to explain the mechanism by which the tetracyclines reduce free fatty acids in skin, that is, by lipase inhibition, further investigation is necessary to support this hypothesis. For example, erythromycin has also been shown to reduce free fatty acids in skin (5), but this antibiotic does not appear to have any effect on pancreatic lipase. It is possible that erythromycin acts solely by its bacteriostatic action, or alternatively, may affect the specific lipases present in comedones without affecting pancreatic lipase. The other drugs tested have no apparent effect on pancreatic lipase and, as far as is known, do not appear to affect free fatty acid formation within the follicle.

Further studies are now in progress in our laboratories to study the effect of these drugs on microbial lipases.

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