2. The maximum hydrolysis of lipin P in mixed lecithin-kephalin preparations corresponded with the percentage content of non-amino (lecithin) N.

3. It is concluded, from the manner of hydrolysis,

that *Cl. welchii* lecithinase hydrolyzes sphingomyelin, as well as lecithin, with production of phosphorylcholine, but does not attack phosphatidylethanolamine nor phosphatidylserine.

REFERENCES

Macfarlane, M. G. (1942). *Biochem. J.* **36**, iii.

Macfarlane, M. G. & Knight, B. C. J. G. (1941). *Biochem. J.* **35**, 884.

Zamecnik, P. C., Brewster, L. E. & Lipmann, F. (1947). *J. exp. Med.* **85**, 381.

The Biochemistry of Bacterial Toxins

3. THE IDENTIFICATION AND IMMUNOLOGICAL RELATIONS OF LECITHINASES PRESENT IN *CLOSTRIDIUM OEDEMATIENS* AND *CLOSTRIDIUM SORDELLII* TOXINS

By MARJORIE G. MACFARLANE, *Lister Institute of Preventive Medicine, London*

(Received 29 October 1947)

Nagler (1939) observed that when *Clostridium welchii* type A was grown in human serum an opalescence developed and eventually a layer of fat rose to the surface. Macfarlane, Oakley & Anderson (1941) found that *Cl. welchii* toxin acts on a clear emulsion of egg yolk to give a similar but more marked opalescence, and that this reaction is due entirely to the α -toxin, the main lethal component of *Cl. welchii* type A culture filtrates; this is termed the lecithovitellin (L.v.) test. Subsequently, *Cl. welchii* toxin was found to contain a lecithinase (Macfarlane & Knight, 1941) which is probably identical with the agent responsible for the Nagler and L.v. reaction, and therefore with the α -toxin.

Hayward (1941, 1943) and Crook (1942) found that other species of *Clostridium*, particularly *Cl. oedematiens*, and also some aerobic spore bearers, may give positive Nagler or L.v. tests; this fact, as pointed out by Hayward (1943), does not militate against the specificity of the test for the detection of *Cl. welchii*, as the essence of the test is not merely a positive reaction but the inhibition of such a reaction by the specific antitoxin. There is, however, a tendency to regard any bacterial agent giving a positive L.v. test as a 'lecithinase', which is clearly unwarrantable without chemical evidence and may lead to confusion on the nature of the bacterial agents.

Macfarlane (1942) briefly reported the identification of a lecithinase in *Cl. oedematiens* toxins, which was immunologically distinct from the main lethal component, and also from *Cl. welchii* lecithinase. Oakley, Warrack & Clarke (1947), in a comprehensive study of the antigenic components of *Cl. oedematiens* toxins, found that the L.v. factor (γ -toxin) in *Cl. oedematiens* type A filtrates was

immunologically distinct from that (β -toxin) present in type B filtrates. Through the courtesy of Dr Oakley, I have been able to show that the immunological behaviour of the lecithinases present in each of these types was similar to that of the L.v. factor, thus indicating that *Cl. oedematiens* β - and γ -toxins are lecithinases. The identification of these lecithinases, and of a lecithinase present in culture filtrates of certain strains of *Cl. sordellii* or *Cl. bifermentans*, is described below.

EXPERIMENTAL

The general methods of procedure and analysis were similar to those used by Macfarlane & Knight (1941). Lecithinase activity was determined by estimation of acid-soluble P liberated from lecithin, and the L.v. factor by the development of turbidity in a clear emulsion of egg yolk.

Toxins and antitoxins

Cl. oedematiens. For the initial experiments in 1942, dry or glycerinated toxins derived from *Cl. oedematiens* 'Albiston' were used. This type B strain toxin was in routine use in the Serum Department of the Lister Institute at Elstree for the hyperimmunization of horses; the various antitoxic sera from Elstree examined for antilecithinase activity were B type (anti- β -toxin). *Cl. oedematiens* type B toxin, O.C. 91044, with corresponding antisera Todd 3 and Todd 61, and type A toxins, A.E. 327, and O.C. 19345 with antisera 202 and 645 were given by Dr Oakley.

Cl. sordellii (or *bifermentans*). Culture filtrates were prepared in this laboratory from five strains (referred to for convenience as *Cl. sordellii*) obtained from Miss Hayward, described as: A 127 A (*sordellii* or *bifermentans*) from necrotic wound; N.C.T.C. 2919, *sordellii*; A 90 b, *sordellii*, from a case of gas gangrene; A 112 (*sordellii* or *bifermentans*); 7851a (*sordellii* or *bifermentans*) from a gangrenous wound.

The strains were subcultured on Robertson's meat medium, and inoculated into the peptone medium described by Macfarlane & Knight (1941). After growth at 37° for 4–18 hr., the fluid was cleared by centrifuging and tested immediately for enzyme activity. The lethality in mice was not tested. *Cl. sordellii* antitoxin was not available.

Expression of results

One lecithinase or enzyme unit (e.u.) is that amount producing 0.1 mg. acid-soluble P from lecithin in 15 min. in standard conditions (Macfarlane & Knight, 1941). One egg yolk unit (y.u.) is that amount producing the same opacity under standard conditions as a test dose of *Cl. welchii* toxin containing one e.u. The unit of *Cl. oedematis* α -antitoxin (anti-lethal) is the International Unit, those for β - and γ -antitoxin are those defined by Oakley *et al.* (1947) with reference to selected sera.

The antilecithinase activity of *Cl. oedematis* antitoxin sera is calculated on the basis that 0.04 unit causes 50% reduction in the hydrolysis under standard conditions by a test dose of *Cl. oedematis* toxin containing 2 e.u. lecithinase. This was adopted because 0.04 unit of *Cl. welchii* antilecithinase (the Elstree Standard no. 1 *Cl. welchii* antitoxin having been arbitrarily selected by Macfarlane & Knight as a standard containing 95 units/ml.) causes approximately 50% reduction in the hydrolysis by 2 e.u. of *Cl. welchii* lecithinase.

These *Cl. welchii* and *Cl. oedematis* antilecithinase units are therefore comparable in a biochemical sense; but since the inhibiting power of the antilecithinase will depend on the total amount of antigen (toxin or toxoid) present in a particular sample of toxin, the *Cl. oedematis* antilecithinase unit is not a fixed standard against all toxins. As the *Cl. oedematis* type A and type B lecithinases are probably identical with the γ - and β -toxins respectively, the antilecithinase activity should preferably be expressed in future in γ - and β -units by comparison of the inhibiting power with that of the standard sera against the same test dose of toxin.

Hydrolysis of lecithin by *Cl. oedematis* toxin

Though the liberation of acid-soluble P from lecithin by *Cl. oedematis* toxin can readily be demonstrated, the quantitative recovery of the hydrolyzed products was more difficult than in *Cl. welchii* hydrolysis, for the low lecithinase activity of the toxins involved either the addition of a large amount of impurities or the prolongation of the hydrolysis, with the risks of autolysis or secondary reactions by associated enzymes.

Identification of products of hydrolysis by type B toxin. The following protocol is representative of a number of hydrolyses.

Egg lecithin (0.5 g. containing 18 mg. P) was emulsified in 15 ml. 0.01 M-CaCl₂, the pH adjusted to 7.4 and 15 mg. *Cl. oedematis* type B toxin (4.5 e.u.) added. The hydrolysis was carried out at 37°, more enzyme being added at 24 hr. and the pH adjusted to 7.4 with NaOH at intervals. After 45 hr., 0.56 mmol. acid had been liberated, equivalent to 95% hydrolysis. The mixture was then separated into

water-soluble and ether-soluble fractions by shaking several times with ether.

The aqueous solution, which contained 91% of the original lipin P as organic P, was evaporated to dryness and the crystalline Ca component of phosphorylcholine obtained from the ethanolic extract of the residue. (Found: P, 9.32; N, 3.7; Cl, 11.4; H₂O, 21.6%. Calc. for C₅H₁₃O₄NCIPCa.4H₂O: P, 9.46; N, 4.25; Cl, 10.75; H₂O, 21.86%.) The yield in the first crop was low, only 25% of that calculated from the organic P liberated; in subsequent hydrolyses the yield of crystalline material was better, up to 60%.

The ethereal solution was evaporated and the residue was extracted with acetone. The dry weight (0.310 g.) of acetone-soluble material was equivalent to an 85% recovery of a diglyceride, calculated as palmitoleylglyceride; it had an acid value 2.7 and saponification value 176 (calculated 179).

Hydrolysis by type A toxin. (1) A lecithin emulsion containing 21.0 mg. lipin P in 0.02 M-CaCl₂ was treated with 20 mg. type A toxin O.C. 19345 at pH 7.4 and 37°, a further 20 mg. enzyme being added after 4 and 20 hr. The hydrolysis was stopped after the liberation of one equivalent of acid and the fatty portion separated from the aqueous solution by extraction with ether. The ethereal solution was evaporated and the residue extracted with acetone, leaving some acetone-insoluble material. The acetone-soluble material (380 mg.), dissolved in ethanol, had a saponification value of 167 but an acid value of 20.

The aqueous solution contained only 15.9 mg. P (76% of the calculated amount) of which 14.1 mg., all as organic P, were recovered on treatment of the evaporated and filtered solution with CaCl₂ and ethanol. All the organic P in a test sample was rapidly hydrolyzed by bone phosphatase, indicating the presence of a monophosphoric ester. The material was redeposited, on addition of ethanol to the aqueous solution, in a more or less crystalline state similar to that of the crystalline Ca compound of phosphorylcholine, but the analysis indicated some impurity. (Found: N, 3.4; Cl, 7.2; P, 8.1%. Calc. for C₅H₁₃O₄NCIPCa.4H₂O: N, 4.25; Cl, 10.75; P, 9.46%.)

(2) The results suggested that the type A toxin contained both a lecithinase splitting off phosphorylcholine, and a lipase attacking either lecithin or the diglyceride formed by the primary action of the lecithinase. A second hydrolysis was carried out on an emulsion containing 17 mg. lipin P, in similar conditions but with 50 mg. toxin initially. The hydrolysis was stopped after 47 hr., when 2.3 equivalents of acid had been liberated. The mixture was acidified with HCl and extracted with ether, the ethereal and aqueous solutions being washed with water and ether respectively.

The aqueous solution contained 13 mg. P (76% of the original lipin P), all in an organic form which was rapidly hydrolyzed by bone phosphatase. A semi-crystalline Ca compound was obtained on treatment of the concentrated solution with ethanol, which had a P:N ratio 1:0.9; the analysis again indicated the presence of some impurity.

The ethereal solution on evaporation gave 314 mg. fatty material, which in ethanolic solution required 4.95 ml. 0.1 N-alkali for neutralization, equivalent to an acid value of 88.5 or approximately 140 mg. free fatty acid as oleic or palmitic acid. The neutral fat and the soap were separated by extraction with ether, and the content of saturated and unsaturated acids in each determined approximately by one separation of the Pb salts in 95% ethanol.

The fatty acids (138 mg.) recovered from the soap gave 85 mg. insoluble Pb salt, equivalent to 60.6 mg. palmitic acid, while 70 mg. fatty acid were recovered from the soluble Pb salt.

The neutral fat (161 mg.) gave 144 mg. acetone-soluble fat, with an acid value less than 4, which was saponified in ethanolic KOH. The saponification value of 159 was approximately that of oleyl or palmityl monoglyceride (156–157); 106 mg. fatty acid were recovered yielding 56 mg. insoluble Pb salts (equivalent to 40 mg. palmitic acid), and 65 mg. fatty acid from the soluble Pb salts.

In both these hydrolyses, therefore, more than 70 % of the lipin P was recovered as a monophosphoric ester with a P : N ratio of approximately unity, and there seems little doubt that *Cl. oedematiens* type A toxin contains a lecithinase similar in action to that of type B toxin and *Cl. welchii* toxin, which decomposes lecithin into phosphorylcholine and a diglyceride. Type A toxin contains in addition a lipase which can split off both saturated and unsaturated fatty acids, either from the diglyceride or possibly from lecithin itself. In the latter case the products of hydrolysis by this second enzyme alone might include a monofatty-acid derivative of glycerophosphorylcholine, as well as glycerophosphorylcholine. The ratio of all the products formed by the toxin would naturally be dependent on the relative activities of the two enzymes, but the difficulty experienced in isolating analytically pure phosphorylcholine suggests that, though the γ -toxin is predominant, traces of other primary products of lecithin were formed.

The identification of two lipolytic enzymes in *Cl. oedematiens* type A toxin—a lecithinase (γ -toxin) similar in action to *Cl. oedematiens* β -toxin and to *Cl. welchii* α -toxin, and a lipase splitting off fatty acids—is consistent with the morphological appearance of type A strains in plate tests and the identification of various components in the culture filtrates by immunological tests. Nagler (1945) showed that the surface of colonies of *Cl. oedematiens* type A, grown

on egg-yolk blood agar, is covered with a 'pearly layer', while type B strains do not produce this effect. Oakley *et al.* (1947) distinguished in type A culture filtrates two components producing opalescence in egg yolk: the γ -toxin and a second factor probably responsible for the 'pearly layer', named ϵ -toxin. It seems reasonable to suppose that the action of the ϵ -toxin is that of the lipase forming a pearly layer of fatty acid upon the colony, but further work is required to identify this enzyme by the inhibitory action of anti- γ and anti- ϵ sera, and to characterize its biochemical nature.

Effect of pH, activators, etc. *Cl. oedematiens* lecithinase (β -toxin) is active over a wide pH range with a broad optimum from 6.0 to 7.6. The pH activity curve was not determined with great accuracy, as it differed somewhat in shape with different toxins, which were all crude.

The enzymic activity was in general increased by the addition of CaCl_2 to the reaction mixture and inhibited by addition of NaF. These crude toxins were, however, active in the absence of added Ca, probably owing to the presence of sufficient Ca in the toxin and lecithin.

Lecithinase content of various toxins. The enzymic activity/unit weight or unit volume was determined by estimation of acid-soluble organic P liberated by measured amounts of the toxin in the following conditions: 1 ml. borate buffer (Palitzsch), pH 7.1; 0.2 ml. 0.3M- CaCl_2 ; 1 ml. 2.5 % aqueous lecithin; toxin or water to 6.0 ml.; incubated at 37° for 15 min.; acidified with 1.0 ml. 10 % trichloroacetic acid and filtered.

Table 1 shows the lecithinase activity of various toxic culture filtrates, or dry toxins prepared from these, together with the lethal capacity (LD_{50}) in mice. The lecithinase activity of *Cl. oedematiens* toxins was very much lower than that of *Cl. welchii* toxins, and varied independently of the main lethal component; *Cl. oedematiens* type A toxins appeared generally to be lower in activity than type B toxins.

Table 1. *Lecithinase activity (e.u.) and lethality (LD_{50}) of Cl. welchii and Cl. oedematiens toxins*

Toxin	Values/mg. dry or ml. fluid toxin		Ratio LD ₅₀ /e.u.
	e.u.	LD ₅₀	
Dry toxins			
<i>Cl. welchii</i> , type A, W.E. 5	12.2	25	2
<i>Cl. oedematiens</i> , type A, A.E. 327	0.06	—	—
<i>Cl. oedematiens</i> , type A, A.E. 208	0.10	—	—
<i>Cl. oedematiens</i> , type A, O.C. 19345	0.15	—	—
<i>Cl. oedematiens</i> , type B, Oe I	0.12	200	1,670
<i>Cl. oedematiens</i> , type B, Oe II	0.30	—	—
<i>Cl. oedematiens</i> , type B, 12 XB	0.29	—	—
Fluid toxins			
<i>Cl. welchii</i> , type A, K. 178	210	400	1.9
<i>Cl. welchii</i> , type A, K. 188	294	450	1.5
<i>Cl. oedematiens</i> , type B, V.F.I	1.5	6,000	3,900
<i>Cl. oedematiens</i> , type B, K.I	1.0	12,000	12,250
<i>Cl. oedematiens</i> , type B, O.C. 91044	1.4	—	—

*Antilecithinase activity of Cl. oedematiens
antitoxic sera*

The general method for measuring the inhibiting action of antitoxic sera was as follows: 1.0 ml. of toxin containing 2 e.u. was allowed to combine with two or three different amounts of antitoxic sera in a total volume of 3.8 ml. for 10 min. at room temperature; borate buffer, CaCl_2 and lecithin were added and the hydrolysis measured in the usual way. The percentage inhibition of the hydrolysis by the same test dose of toxin without antitoxin in the same test was plotted against the amount of antitoxin, the amount equivalent to 50% inhibition being regarded as containing 0.04 antilecithinase units. Using homologous antitoxins which produced complete inhibition in excess, an approximately linear relationship obtained in the above conditions.

Table 2. *Antilecithinase titres of Cl. oedematiens type A and type B antisera against type B and type A lecithinase*

(1 e.u. of toxin A.E. 327 = 17 mg. $\equiv$ 8 test doses for L.V. test. 1 e.u. of toxin O.C. 91044 = 0.7 ml. $\equiv$ 31 test doses for L.V. test.)

Serum	Antilecithinase units/ml. serum against 1 e.u. test toxin		Antitoxin titres (L.V. units/ml.)	
	Type A toxin A.E. 327	Type B toxin O.C. 91044	γ -units	β -units
Type A 202	5.0	< 0.2	700	0.5
644	7.7	—	700	2.0
Type B Todd 3	0.4	40	120	2500
Todd 61	0.2	—	65	450

A number of type B antitoxic horse sera prepared by routine immunization, and containing from 750 to 2500 international units/ml., were tested against the type B toxin Oe II and found to contain small amounts of antilecithinase (0.2–3.0 units/ml.). The ratio i.u./antilecithinase units varied from 250 to 10,000; thus the antilecithinase is clearly distinct from the anti-lethal toxin (Oakley's α -antitoxin).

Immunological specificity of type A and type B antilecithinase. The cross reactions between type A and type B antitoxic sera and type B and type A lecithinases were tested in a similar manner, but with a lower test dose of toxin (1 e.u.) owing to the small amounts available. The approximate antilecithinase titres against the same number of e.u.'s of the homologous and heterologous lecithinases are shown in Table 2, together with the titres for β - and γ -antitoxin determined by Dr Oakley. There is clearly a marked difference between the inhibiting power against the homologous and the heterologous enzyme. Considering the limits of accuracy of the tests, and the different basis of testing, there is also a substantial agreement between the ratio of the antilecithinase titre of a particular serum against the homologous and the heterologous toxin, and the

ratio of its β - and γ -antitoxin content. It seems fair to conclude that the type A and type B lecithinases are identical respectively with the γ - and β -toxins distinguished by Oakley *et al.* (1947).

A protocol (Table 3) shows that, while there was practically no inhibition of the β -lecithinase by the type A serum, there was complete inhibition of the γ -lecithinase by the type B serum (Todd 3) if sufficient serum was added. Inspection of the titres for various sera given by Oakley *et al.* (1947, Table 8) shows that the ratio γ/β -units in a β -rich serum was always considerably higher than the ratio β/γ -units in a γ -rich serum. This is probably not a genuine cross reaction between β -antitoxin and γ -lecithinase, but is due simply to the fact that all the horses had received a preparatory dose of alum-precipitated toxoid from a type A (γ -toxin) filtrate; it is at least unlikely that complete inhibition in the lecithinase test, which in contrast to the egg-yolk

test is augmented rather than inhibited by non-specific protein, could be due to non-specific inhibition by a low-titre serum.

Table 3. *Inhibition of type A lecithinase by type B antisera*

Toxin	Antiserum (ml.)	Hydrolysis (% of test dose)
Type A		
Test dose 1 e.u. γ -lecithinase	Type A 0.004	68
	0.01	32
	0.02	10
	Type B 0.007	96
	0.033	79
	0.10	49
	0.20	16
Type B		
Test dose 1 e.u. β -lecithinase	Type A 0.004	94
	0.02	93
	Type B 0.001	55
	0.007	0
	0.02	0

Species specificity of Cl. welchii and Cl. oedematiens lecithinase. The cross reactions of *Cl. welchii* type A and *Cl. oedematiens* type B antitoxins with the

heterologous enzymes were examined in a similar way. There was practically no inhibition of either of the enzymes by the heterologous antitoxin, in amounts greatly exceeding those inhibiting the homologous enzyme.

Lecithinase activity of Cl. sordellii culture filtrates

Egg-yolk and lecithinase tests. Filtrates of 23 hr. cultures of five strains of *Cl. sordellii* were tested for their capacity to produce turbidity in a clear emulsion of egg yolk, in comparison with a test dose (1 e.u.) of *Cl. welchii* toxin. Three of the strains gave a positive reaction (N.C.T.C. 2919, A90B, A112) with values 1.0, 0.9 and 0.5 y.u./ml. The first two filtrates were tested for lecithinase by estimation of acid-soluble P liberated from lecithin, and gave values of 0.7 e.u./ml. In various tests on culture filtrates from these strains, the lecithinase activity was lower, to a variable extent, than the egg-yolk value, indicating the presence of a second factor affecting egg yolk. As the routine test for lecithinase was complicated by the presence of inorganic P in a culture filtrate of low activity, an attempt was made with one filtrate to purify the enzyme by salting it out, but the dialyzed solution showed an even greater discrepancy between the egg factor (3 y.u./ml.) and the lecithinase (0.9 e.u./ml.).

Decomposition of egg yolk. The decomposition of egg yolk by a *Cl. sordellii* filtrate and by *Cl. welchii* toxin was followed with reference both to the conversion of lipin P to an acid-soluble form, and to the liberation of acid-soluble N. Table 4 shows that almost all the egg-yolk P, including presumably the bound lipin P as well as the ether-soluble P, was converted into acid-soluble organic P; there was slightly more formation of acid-soluble N by *Cl. sordellii* than by *Cl. welchii* toxin. It is possible that *Cl. sordellii* cultures contain an enzyme attacking the yolk protein and thereby affecting the turbidity development in the egg-yolk test, but it seems more probable that this toxin, like the *Cl. oedematiens* type A toxin, contains a lipase, for *Cl. sordellii* also gives a 'pearly layer' in plate tests. The products of hydrolysis of lecithin by *Cl. sordellii* toxin were not identified, but the action of the lecithinase is

apparently similar to that of *Cl. welchii* in so far as an acid-soluble phosphoric ester is produced.

Inhibition by heterologous antitoxic sera. Owing to their low lecithinase activity, the culture filtrates from these strains of *Cl. sordellii* were not suitable for tests of the antilecithinase activity of sera, but some tests were carried out with the egg-yolk test. With one filtrate, inhibition either by *Cl. welchii* or *Cl. oedematiens* antitoxic sera was maximal at 50%; with other filtrates complete inhibition was sometimes observed. Further work is required on purified and concentrated preparations of *Cl. sordellii* lecithinase to determine whether this inhibition is a true partial inhibition of the lecithinase, or a specific inhibition of a second factor affecting egg yolk, or a non-specific inhibition of the development of turbidity by the excess protein added. So far as it goes, the result is in accord with Hayward's (1943) finding in plate tests, that the Nagler reaction of some strains of *Cl. bifermentans* or *Cl. sordellii* is completely inhibited, and that of other strains partially inhibited, by *Cl. welchii* antitoxin.

DISCUSSION

It has been shown previously that the lecithinase present in *Cl. welchii* toxin is probably identical with *Cl. welchii* α -toxin, the main lethal, necrotic and haemolytic component of *Cl. welchii* type A toxins. Evidence has now been presented that culture filtrates of *Cl. oedematiens* contain a lecithinase similar in biochemical action to *Cl. welchii* lecithinase, but quite distinct from the main lethal component. The lecithinases present in culture filtrates from *Cl. oedematiens* type A and type B strains are immunologically distinct from each other, and there seems little doubt that they are identical respectively with the γ - and β -toxins described by Oakley *et al.* (1947). These authors point out that, as lecithinases, β - and γ -toxins might be expected to be haemolytic, necrotic and lethal, and that their own failure to demonstrate a lethal effect with certainty, after adsorption of the main lethal component with an appropriate antitoxic serum, was probably due to the small amount of lecithinase present. Assay of the lecithinase activity of various *Cl. oedematiens*

Table 4. *Liberation of P and N from egg yolk by Cl. welchii and Cl. sordellii toxins*

(Reaction mixture: 10 ml. yolk emulsion (1.1 mg. total P) and 2 ml. toxin (2 e.u.) or saline; 4 hr. at 37°.)

Determination	Found after incubation (mg.)		
	Control, without toxin	With <i>Cl. welchii</i> toxin	With <i>Cl. sordellii</i> toxin
P in ether-soluble fraction	0.56	Nil	Nil
Ortho P in trichloroacetic acid-soluble fraction	Nil	Nil	Nil
Total P in trichloroacetic acid-soluble fraction	Nil	0.96	0.97
N in trichloroacetic acid-soluble fraction	Nil	0.90	1.35

filtrates shows that this explanation is probably correct, since the maximum content found (1.5 e.u./ml.) is only equivalent (by comparison, with *Cl. welchii* lecithinase) to approximately 3 LD₅₀ doses/ml. for the mouse, compared with 6000–1200 LD₅₀ doses/ml. of the main lethal component (*Cl. oedematiens* α -toxin).

The immunological and enzymic specificities of the lecithinases present in *Cl. welchii* and *Cl. oedematiens* toxins offer some entertaining facts for those interested in the relationship between chemical configuration and biological action. It may be supposed that the group responsible for lecithinase activity is closely similar in the three enzymes (*Cl. welchii* α -toxin, *Cl. oedematiens* β - and γ -toxins); the fine configuration of *Cl. welchii* lecithinase is such that it decomposes lecithin and, probably but less readily, sphingomyelin, but not kephalin (Macfarlane, 1942, 1948) while the failure to attack lysolecithin and glycerophosphorylcholine (Zamecnik, Brewster & Lipmann, 1947) suggests that the presence of two fatty acids is essential for orientation of the substrate molecule. Each of these enzymes is immunologically distinct; since the antilecithinase groups of the specific antibodies are presumably as similar to each other as the lecithinase groups of the antigens, it may be inferred that the immunological distinction between these antibodies is conferred by a 'species' or 'type' group in the antigen. But in other instances the immunological distinction is presumably conferred by the enzymic or 'toxic' group of the antigen, e.g. the distinction between the antilecithinase, antihyaluronidase, anticollagenase, etc. of *Cl. welchii* antitoxic sera. Thus these bacterial enzymes carry in effect two specific antigenic configurations, e.g. 'W + lecithinase' or 'Oe A + lecithinase'; whether these configurations are discrete or whether they are interlocked to form a unique configuration, the structure is such that it can combine specifically both with the substrate of the enzyme, and with a type-specific anti-enzyme.

The immunological specificity of these 'toxic' enzymes is no doubt a reflexion of the stereochemical obtrusiveness of the antigenic structure, which is not

without bearing on the virulence of the toxins. Discussing the behaviour of toxic enzymes, Macfarlane & Knight (1941) said, 'The toxin can primarily affect only those cells which contain the appropriate substrate for its enzymic activity, and then only if this substrate is accessible to it'. The very interesting fact has been pointed out by Oakley *et al.* (1947) that sheep red cells are readily attacked by the lecithinase *Cl. welchii* α -toxin, but are insensitive to the lecithinases *Cl. oedematiens* β - and γ -toxins; the latter haemolyze horse red cells, which are not attacked by *Cl. welchii* α -toxin. One possible explanation of this phenomenon is that stereochemically the specific blood-group substance of the host permits or hinders the approach of the type-specific group of the lecithinase, and thus permits or prevents the orientation of the enzyme towards the lecithin of the red-cell membrane. A similar kind of interference may well be the basis of the relative 'susceptibility' or 'resistance' of different host species to a particular toxin. It is clear that the activity of a bacterial enzyme towards a biological structure cannot be predicted merely on the basis that the structure contains the necessary substrate.

SUMMARY

1. *Cl. oedematiens* culture filtrates contain a lecithinase similar in biochemical properties to that present in *Cl. welchii* filtrates.
2. The lecithinases present in *Cl. oedematiens* type A and type B filtrates are immunologically distinct from each other, and from *Cl. welchii* lecithinase, in that their activity is inhibited by a homologous but not inhibited by a heterologous antitoxic serum. The type A and type B lecithinase are probably identical respectively with the γ - and β -toxins of *Cl. oedematiens* filtrates.
3. *Cl. oedematiens* type A toxins also contain a lipase, which is possibly identical with the factor (ϵ -toxin) responsible for the 'pearly layer' effect in colonies of this organism.
4. Culture filtrates of certain strains of *Cl. sordellii* also contain a lecithinase which is not yet characterized.

REFERENCES

- Crook, E. M. (1942). *Brit. J. exp. Path.* **23**, 37.
 Hayward, N. J. (1941). *Brit. med. J.* **1**, 811.
 Hayward, N. J. (1943). *J. Path. Bact.* **55**, 285.
 Macfarlane, M. G. (1942). *Biochem. J.* **36**, iii.
 Macfarlane, M. G. (1948). *Biochem. J.* **42**, 587.
 Macfarlane, M. G. & Knight, B. C. J. G. (1941). *Biochem. J.* **35**, 884.
 Macfarlane, R. G., Oakley, C. L. & Anderson, C. G. (1941). *J. Path. Bact.* **52**, 99.
 Nagler, F. P. O. (1939). *Brit. J. exp. Path.* **20**, 473.
 Nagler, F. P. O. (1945). *Aust. J. exp. Biol. med. Sci.* **23**, 59.
 Oakley, C. L., Warrack, G. H. & Clarke, P. H. (1947). *J. gen. Microbiol.* **1**, 91.
 Zamecnik, P. C., Brewster, L. E. & Lipmann, F. (1947). *J. exp. Med.* **85**, 381.