REFERENCES

- Cohen, S. (1951). *J. Immunol.* **67**, 339.
 Cohn, E. J., McMeekin, T. L., Oncley, J. L., Newell, J. M. & Hughes, W. L. (1940). *J. Amer. chem. Soc.* **62**, 3386.
 Coombs, R. R. A., Mourant, A. E. & Race, R. R. (1945). *Brit. J. exp. Path.* **26**, 255.
 Coombs, R. R. A. & Race, R. R. (1945). *Nature, Lond.*, **156**, 233.
 Dacie, J. V. (1950). *Nature, Lond.*, **166**, 36.
 Davis, B. D., Moore, D. M., Kabat, E. A. & Harris, A. (1945). *J. Immunol.* **50**, 1.
 Deutsch, H. F., Alberty, R. A., Gosting, L. J. & Williams, J. W. (1947). *J. Immunol.* **56**, 183.
 Francis, G. E., Mulligan, W. & Wormall, A. (1951). *Nature, Lond.*, **167**, 748.
 Gleeson-White, M. H., Heard, D. H., Mynors, L. S. & Coombs, R. R. A. (1950). *Brit. J. exp. Path.* **31**, 321.
 Kabat, E. A. (1939). *J. exp. Med.* **69**, 103.
 Kabat, E. A. & Mayer, M. M. (1948). *Experimental Immunochimistry*, p. 77. Springfield, Illinois: Charles C. Thomas.
 Kekwick, R. A. (1940). *Biochem. J.* **34**, 1248.
 Masouredis, S. P., Melcher, L. R. & Koblick, D. C. (1951). *J. Immunol.* **66**, 297.
 Pressman, D. & Sternberger, L. A. (1950). *J. Amer. chem. Soc.* **72**, 2226.
 Race, R. R. (1944). *Nature, Lond.*, **153**, 771.
 Race, R. R. & Sanger, R. (1950). *Blood Groups in Man*, p. 82. Oxford: Blackwell.
 Wintrobe, M. M. (1942). *Clinical Hematology*, p. 200. London: Henry Kimpton.
 Wiener, A. S. (1944). *Proc. Soc. exp. Biol., N.Y.*, **56**, 173.

The Chemical Composition of the Soluble and Insoluble Fractions of the Bacterial Cell

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Investigation of the chemistry of the bacterial cell wall has been retarded by lack of suitable methods. The results of immunological investigations have suggested that in some organisms its structure is complex, with successive layers of different antigens. Another approach has been through the extraction of whole-cell preparations with organic solvents. Morgan & Partridge (1940) and Freeman, Challinor & Wilson (1940) have extracted polysaccharide-protein complexes from certain Gram-negative bacteria. These substances accounted for about 10% of the whole cell and were believed on immunological evidence to comprise the outer layers. Henry & Stacey (1946) extracted Gram-positive bacteria with bile salt and obtained a magnesium ribonucleate which they considered to be a surface component and responsible for the retention of the Gram stain. Caution must be exercised in interpreting the results of these extraction experiments since it is difficult to establish from which part of the cell the extracted material has come.

Curran & Evans (1942) and King & Alexander (1948) killed bacteria by prolonged shaking with minute glass beads. Dawson (1949) showed that this treatment ruptures the cell wall and allows the contents to escape, leaving the empty shell more or

less intact. This seemed to offer the possibility of separating cell walls for chemical study. Salton & Horne (1951*a, b*) separated cell-wall material by this method, and also by heat treatment and by treatment with alkali. More recently, Salton (1952*a*) has found that the cell wall of *Streptococcus faecalis* is composed of a protein-polysaccharide complex, similar, perhaps, to those obtained by extraction methods from the Gram-negative bacteria (Morgan & Partridge, 1940; Freeman *et al.* 1940). He suggests that mucoproteins may be important components of bacterial cell walls. Salton (1952*b*) finds that the lipid content of Gram-positive cell walls is low (1–2%) compared with that of Gram-negatives (20%), and there are also differences in the amino acids of the protein component. Mitchell & Moyle (1951) find that three-quarters of the cell-wall material of *Micrococcus pyogenes* is accounted for by a glycerophosphoprotein complex which they regard as being probably characteristic of Gram-positive types. Holdsworth (1952) finds that the 'insoluble fraction' of *Corynebacterium diphtheriae* contains 60% protein and 25% carbohydrate. Northcote & Horne (1952) have described certain polysaccharides from yeast cell walls.

METHODS

Organisms. Four Gram-negative types were studied (*Escherichia coli* E106; *Pasteurella pseudotuberculosis* N.C.T.C. 1102; *Proteus vulgaris* L10; *Pseudomonas aeruginosa* L15); three Gram-positive types (*Bacillus subtilis* E4; *Micrococcus pyogenes*, var. *albus* (*Staphylococcus albus*) L14; *Sarcina lutea* L21); and one acid-fast organism (*Mycobacterium phlei*, N.C.T.C. 525). *Myco. phlei* and *Past. pseudotuberculosis* were obtained from the National Collection of Type Cultures: the other organisms were stock laboratory strains and the numbers refer to our own list.

Media. Glucose broth: a peptone-Lemco-yeast extract broth (Oxo Dried Medium, CM1, Oxo Ltd., Thames House, London, E.C. 4), with addition of 0.5% glucose. The Oxo medium was made up in the appropriate volume of tap water and sterilized (5 lb./30 min.). The glucose was prepared as an acidified 50% (w/v) solution, sterilized (5 lb./30 min.) and the appropriate volume added aseptically to the broth.

Glucose agar: glucose broth solidified with 2% B.D.H. agar powder.

Stock cultures were maintained on glucose-agar. For each experiment five Roux bottles, each containing 150 ml. glucose agar, were inoculated with 5 ml. of a 24 hr. glucose-broth culture of the organism under examination. After incubating for 19 hr. at 37° the organisms were washed off with water, passed through a coarse sintered-glass filter to remove particles of agar, centrifuged, washed three times on the centrifuge, and suspended in 10 ml. water. The yield was about 0.5 g. dry weight in most cases. The organisms were 'mature', i.e. they had been harvested shortly after completion of the phase of logarithmic growth.

Disintegration of organisms

The 10 ml. suspension was placed in a 1 oz. screw-cap bottle with 10 g. of grade 12 Ballotini (Chance Bros., Smethwick, England); the bottle was shaken for 4 hr. along its long axis, at about 750 strokes/min. with a 2.5 in. stroke in a machine similar to that used by King & Alexander (1948). The bottles were packed in ice during shaking, and the temperature of the contents did not exceed 10°. The shaker accommodated two bottles and all runs were done in duplicate. Viable counts were made before and after shaking, and in all cases a reduction to less than 10⁻⁴ of the original value was obtained. Microscopic examinations were also made. With *B. subtilis*, for example, the unshaken organisms were clearly visible under dark-ground illumination and highly refractile. After 0.5 hr. shaking, most of the cells appeared to be broken; usually the end of the bacillus seemed to have been broken off. The broken cells were also easily distinguished as they were not highly refractile. Some cells still retained the Gram stain: the remainder stained only weakly. After 1 hr., when the viable count had already, in this case, fallen below 10⁻⁴ times its initial value, there were very few intact cells and an appreciable amount of amorphous debris in the form of particles on the limit of visibility of the dark-ground microscope. The Gram staining reaction was almost negative. Further shaking led to an increase in the amount of debris. These observations were made on the bulk of the fluid in the vessel: the froth which formed during shaking contained rather more intact cells.

After shaking, the disintegrated material was poured through a coarse (grade 1) sintered-glass filter, which separated off the Ballotini. The Ballotini were then washed on the filter with 5 ml. lots of water until the runnings were quite clear. The total volume (material plus washings) was now 30–40 ml. Harvesting and disintegration was a full day's work and the material was frozen solid at this stage and left overnight. After thawing, the disintegrate was centrifuged for 10–15 min. at 10000 rev./min. (14000 × g) to yield an almost clear supernatant. This was carefully decanted off and the insoluble fraction was resuspended in 15–20 ml. water with very thorough shaking, and recentrifuged. This washing procedure was carried out three times: the absence of any frothing in the supernatant then showed that no more soluble protein material was being washed out. The washings were combined with the original supernatant and made up to 100 ml. This was the 'soluble fraction' of the cell. The insoluble material was suspended in 20 ml. water and a homogeneous suspension obtained by vigorous mechanical shaking. Any extraneous matter was removed by passing through a coarse sintered-glass filter, and the filter washed, and the runnings made up to a total volume of 25 ml. This was the 'insoluble fraction'. During all centrifuging procedures the centrifuge cups were sealed with Sellotape: otherwise sufficient foreign matter found its way in to cause serious errors.

Analytical methods

Dry weight. This was determined by freeze-drying a suitable sample (5 ml. insoluble or 25 ml. soluble fraction).

Ash content. A suitable volume of the solution or suspension was freeze-dried in a nickel crucible and incinerated at 600° in an electric muffle furnace. Errors in ash content arising from abrasion of the beads were small. In one experiment 490 mg. (dry weight) of organism yielded 42.5 mg. ash before disintegration, 43 mg. after treatment. The increase in ash content was thus within the experimental error of determination. On the other hand, very serious errors in ash content may arise if the centrifuge cups are not covered during centrifugation.

Total nitrogen by Kjeldahl. The incineration technique of Chibnall, Rees & Williams (1943) and the distillation apparatus of Markham (1942) were used.

Phosphate. Phosphate was determined by the method of Berenblum & Chain (1938).

Hexose and pentose. These were estimated simultaneously by the differential colorimetric method of Fernell & King (1953).

Deoxyribose. This was determined by Dische's (1930) method. The reaction could not be carried out with deeply coloured materials such as the insoluble fraction of *Ps. aeruginosa*. The insoluble fractions of other organisms also gave trouble on account of turbidity. This was overcome as follows. The material was heated with the Dische reagent in the usual way, and then filtered through a very thin layer of washed talc supported on a fine (5/3) sintered-glass filter. The talc did not remove any of the colour, nor were the fine particles filtered off by the talc coloured. As no deoxyribose was available for use as a standard, a commercial sample of thymus nucleic acid was used; it gave correct analytical figures for N and P, and was free of pentose. This was convenient, since it was assumed that the deoxyribose present would be in the form of deoxyribonucleic acid (DNA), and results are expressed in terms of DNA.

Total nucleic acid. Mitchell (1950) describes a method for the spectroscopic estimation of nucleic acid in bacteria based on the work of Malmgren & Heden (1947). The absorption of a suspension is measured at 260 m μ . and a correction applied for scattering. A difficulty arises in the selection of the correct factor for the absorption coefficient. Mitchell takes $E_{1\text{cm}}^{1\%}$ 200, which he regards however as a minimum value. A figure of 250 accorded better with our own measurements on both thymus and yeast nucleic acids. We found no change in the specific absorption at 260 m μ . when organisms were disintegrated, and concluded that the absorption coefficient does not change when nucleic acids are liberated from the cell. Although, as Mitchell admits, 'the method depends on certain assumptions of questionable validity', it is valuable for making comparative observations such as those with which the present study is concerned. We found it unsuitable however for the insoluble fractions. Absorption, or scattering, rises very rapidly as the wavelength decreases. Some organisms show no break at all at 260 m μ ., others only a slight inflexion. Whether the steep rise observed at the shorter wavelengths is due to non-specific absorption or to anomalous scattering we do not know. Mitchell's correction for scattering is based on the assumption that the scattering particles were bacterial cells, and is not necessarily valid when dealing with smaller particles of very varying size.

RESULTS

The possibility of changes in the cell constituents during treatment was considered, although the temperature was kept below 10° during disintegration. A suspension of *Esch. coli* was therefore disintegrated and samples withdrawn at intervals for assay. Values for nucleic acid, deoxypentose, pentose, and hexose did not differ significantly from those for the untreated organism. The absorption spectrum revealed a considerable fall in scattering in the first half-hour but no change in the specific nucleic acid absorption.

Composition of fractions

Two suspensions, prepared from separate cultures of each organism, were disintegrated and assayed separately. The two series of replicate results are listed under 'culture 1' and 'culture 2' in the tables. The proportion of the whole cell accounted for by the insoluble fraction is given in Table 1, and the analytical figures for the separate fractions in Table 2.

Table 1. *Insoluble fraction of disintegrated bacteria expressed as percentage dry weight of whole organism*

	Culture 1	Culture 2
<i>B. subtilis</i>	53.7	54.7
<i>Sarc. lutea</i>	45.9	47.6
<i>M. pyogenes</i>	40.5	41.6
<i>Esch. coli</i>	37.1	39.6
<i>Ps. aeruginosa</i>	76.6	78.0
<i>Pr. vulgaris</i>	29.4	20.8
<i>Past. pseudotuberculosis</i>	64.1	65.5
<i>Myc. phlei</i>	51.9	51.7

Table 2. *Analysis of insoluble and soluble fractions of disintegrated bacteria*

(All results expressed as percentage dry weight of fraction concerned.)

	<i>B. subtilis</i>		<i>Sarc. lutea</i>		<i>M. pyogenes</i>		<i>Esch. coli</i>		<i>Ps. aeruginosa</i>		<i>Pr. vulgaris</i>		<i>Past. pseudotuberculosis</i>		<i>Myc. phlei</i>	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Insoluble	3.7	3.9	10.0	10.7	8.4	7.7	8.0	8.5	3.60	3.72	8.5	7.9	3.6	3.3	9.8	10.3
Soluble	12.5	12.5	12.5	11.2	14.8	13.2	7.9	7.9	7.8	7.2	8.0	7.9	7.3	7.1	7.8	8.1
Insoluble	8.7	8.7	6.1	5.6	9.9	10.1	9.5	9.6	10.0	10.3	9.6	9.4	5.6	5.8	6.9	6.3
Soluble	7.9	7.6	8.0	8.9	11.0	10.0	12.9	12.2	9.3	9.3	11.3	11.1	7.8	7.9	7.7	8.0
Insoluble	0.47	0.44	0.28	0.19	1.74	1.76	0.85	0.85	0.99	1.02	0.37	0.34	0.22	0.23	0.14	0.15
Soluble	0.65	0.65	1.67	1.63	2.4	2.1	1.93	2.00	3.0	2.9	0.96	0.93	1.80	1.75	1.26	1.30
Insoluble	2.58	2.41	10.9	10.2	3.8	3.7	10.3	10.9	5.0	6.8	7.7	7.8	2.33	1.80	4.7	4.8
Soluble	15.1	16.1	5.9	8.0	6.0	5.0	10.2	11.6	6.6	6.6	3.1	2.9	14.8	14.4	6.4	6.2
Insoluble	0.85	0.85	0.95	0.90	0.95	1.24	1.50	1.39	2.3	2.0	1.74	1.83	0.14	0.14	4.4	3.3
Soluble	3.8	4.1	1.72	1.70	7.7	6.5	4.4	4.2	4.3	4.4	3.7	3.4	3.1	2.9	6.4	6.2
Insoluble	0.28	0.28	0.39	0.33	0.26	0.26	1.75	1.81	(Not assayed)		1.28	1.28	0.69	0.75	0.77	0.78
Soluble	1.26	1.24	1.96	1.98	2.78	2.35	5.07	5.25	14.1	14.3	6.0	6.4	2.32	2.05	7.6	7.8
Soluble	16.1	16.9	11.8	11.7	15.1	12.8	20.3	20.7	25.8	25.8	20.0	18.6	19.6	17.3	16.4	15.9

Deoxyribonucleic acid
Total nucleic acid

The ash content of the soluble fraction is higher in the Gram-positive than in the Gram-negative organisms. In all cases the soluble fraction is richer in phosphorus than the insoluble fraction.

Carbohydrates

The pentose content is higher, usually much higher, in the soluble fraction. This is probably due to the very low nucleic acid content of the insoluble material. Owing to the stability of the pyrimidine nucleotides to acid hydrolysis (Schneider, 1945) only 45 % of the ribonucleic acid pentose will be estimated. In the soluble fraction which has a high nucleic acid content, the figures given may be only half the true value. The precise correction to be applied cannot be stated since no detailed analysis of the nucleic acids was made. 'Pentose' does not include deoxypentose. Amino sugars do not assay in the methods used.

Holdsworth (1952) found 2.0 % pentose in the soluble fraction of *C. diphtheriae* and 9.7 % in the insoluble fraction, but the cell wall of this organism contains a polysaccharide with an arabinose unit.

The three Gram-positives are relatively poor in deoxypentose, especially in the insoluble fraction.

Nucleic acid

Data are given for the soluble fraction only. Absorption spectra of both fractions were examined in all cases. In the insoluble fractions scattering was high and the photometer reading increased rapidly as wavelength decreased, with either no detectable break in the curve at 260 m μ . or at most only a slight inflexion. Even when scattering is reduced by hydrolysis with acid, the spectra still suggest that no appreciable amounts of nucleic acid are present. This is in agreement with Salton & Horne's (1951b) findings for the cell wall of *Strep. faecalis*. The soluble fractions, on the other hand,

Fig. 1. Absorption spectra of soluble fractions; *Esch. coli*, 0.120 mg./ml. (continuous line); *B. subtilis*, 0.136 mg./ml. (broken line). Measured in 1 cm. cell in Beckman spectrophotometer.

gave absorption spectra characteristic of nucleic acid. Fig. 1 gives the curves obtained with *B. subtilis* and *Esch. coli*; the other organisms gave curves similar in form but differing in the height of the 260 m μ . peak.

Effect of medium

The composition of a bacterial cell may be much influenced by the medium in which it is grown. No detailed investigations were made of the relative effect of the medium on the two fractions, but in one experiment we compared the composition of the fractions from cultures of *B. subtilis* and *Esch. coli* grown both on our standard glucose-agar medium, and on a similar medium without glucose.

The results (Table 3) show that on the whole the soluble fraction is more affected by this change in the medium.

Table 3. Influence of medium on composition of fractions

(*Esch. coli* and *B. subtilis* were grown on (a) plain agar and (b) 0.5 % glucose agar. The table gives the yield of the insoluble fractions expressed as % dry wt. of the whole cell and the components of the various fractions as % dry wt. of the fraction concerned.)

	<i>Esch. coli</i>		<i>B. subtilis</i>	
	Glucose	No glucose	Glucose	No glucose
Insoluble fraction (% dry wt. of whole cell)	42	37	55	43
N, insoluble fraction	9.5	8.8	8.8	12.6
N, soluble fraction	12.5	11	7.5	10
P, insoluble fraction	0.84	1.28	0.45	0.45
P, soluble fraction	1.94	1.84	0.54	1.84
Pentose, insoluble fraction	1.5	1.5	0.9	0.7
Pentose, soluble fraction	4.6	1.9	4.0	2.5
Hexose, insoluble fraction	10.6	11.9	2.5	5.4
Hexose, soluble fraction	10.9	2.3	15.8	1.2
Deoxyribonucleic acid, insoluble fraction	1.75	2.42	0.28	1.04
Deoxyribonucleic acid, soluble fraction	5.6	2.64	1.3	1.48
Total nucleic acid, soluble fraction	20.4	16.4	16.4	12.6

DISCUSSION

Cooper, Rowley & Dawson (1949) criticize the disintegration technique on the grounds that up to 7% of the cells escape destruction, and Salton & Horne (1951*b*) claim only that 'over 90%' were disintegrated. Mitchell & Moyle (1951) found less than 1% of cells of *M. pyogenes* intact. These authors all used the Mickle disintegrator with a higher speed but a much shorter stroke than our shaker. Previous work (King & Alexander, 1948) showed that appropriate shaking technique would destroy nearly all the cells, and King & Stein (1950) achieved complete sterilization of even thick suspensions of *B. anthracis*. We found, in addition to the factors discussed by King & Alexander, that the proportion of the vessel occupied by the suspension plus beads is important. Destruction is much retarded if an appreciable air space is not left in the vessel (Fig. 2).

Dawson (1949), Salton & Horne (1951*b*) and Mitchell & Moyle (1951) have examined, by electron microscopy, materials corresponding to our 'insoluble fraction'. They observed no formed elements other than those derived from the cell wall. Mitchell & Moyle obtained two fractions on centrifugation of their disintegrated bacteria,

Fig. 2. Effect of volume of contents of vessel on disintegration of organisms. A thick suspension of *Esch. coli* was shaken in a 1 oz. bottle completely filled with the suspension and beads (●—●) and also in a bottle only half filled (○—○). Samples (0.5 ml.) were withdrawn at hourly intervals and the viable count determined. With the full bottle the rate of destruction was relatively low until, after 4 hr., sufficient fluid had been withdrawn for the purpose of making the counts to leave an appreciable air space in the vessel.

a lower layer containing only recognizable cell-wall fragments and an upper layer containing finer particles as well. They conclude, however, that the latter (in view of their chemical composition) were also derived from the cell wall. Our material includes both their fractions. Although we have not been able to submit our insoluble fraction to the final test of electron microscopy, it is clear that it is derived mainly, if not entirely, from the cell wall. The cell wall has hitherto been generally regarded as a relatively thin outer layer, by analogy with the cell wall of plants. It is therefore surprising to find that the insoluble fraction comprises so large a portion of the whole cell. Nevertheless, this agrees with the findings of others, e.g. 25% for *M. pyogenes* (Mitchell & Moyle, 1951); 57% for *C. diphtheriae* (Holdsworth, 1952). In a coccus, say, 0.8 μ . in diameter, a wall only 80 m μ . thick would occupy half the volume: Dawson (1949) obtained a value of 15–20 m μ . by direct measurement of a cell-wall preparation of *M. pyogenes* which had however been subjected to the intensive drying required for electron microscopy. Evidently, the cell wall separated by the disintegration procedure, i.e. our insoluble fraction, represents a substantial outer zone of the cell rather than the classical idea of a cell wall, and the term cell wall may require redefinition as far as bacteria are concerned.

The most striking difference between the insoluble, or outer-zone fraction, and the soluble fraction is the paucity of nucleic acid in the former. The characteristic absorption at 260 m μ . is absent and the figures for phosphorus, pentose and deoxypentose are lower (in most cases much lower) than those for the soluble fraction. Apart from this, the chemical composition of the insoluble fraction shows no clear pattern common to all species. This is hardly surprising in view of the variety of materials reported as characteristic of the cell wall. Two quite different substances have indeed been claimed as the characteristic surface component of the Gram-positive group, namely, the magnesium ribonucleate of Henry & Stacey (1946) and the polyolphosphate, or phosphate ester of an unidentified polyhydric alcohol, of Mitchell & Moyle (1951). Henry & Stacey, however, did not demonstrate unequivocally that their material was derived from the cell wall, whilst Mitchell & Moyle worked with *M. pyogenes*, an organism whose insoluble fraction is exceptionally rich in phosphorus. Holdsworth (1952) found that 45% of the cell wall of the Gram-positive *C. diphtheriae* consisted of an oligosaccharide-protein complex. The mucoprotein described by Salton (1952*a*) in *Strep. faecalis*, like the similar substances from Gram-negative types (Morgan & Partridge, 1940; Freeman *et al.* 1940) cannot be the main cell-wall component as it accounts for less than 10% of the whole cell.

SUMMARY

1. The soluble and insoluble fractions of eight species of bacteria were separated by centrifuging the material obtained after disintegrating the cells by shaking with glass beads.

2. The insoluble material, believed to be at least mainly derived from the cell wall, accounted in most cases for about half of the dry weight of the cell.

3. Estimations were performed on both fractions for ash, nitrogen, phosphorus, hexose, pentose, deoxypentose and nucleic acid, the last being determined spectroscopically.

4. Wide variations were found among different organisms but certain regularities were observed. Notably, nucleic acid was confined to the soluble fraction, which was also much richer in phosphorus, pentose and deoxypentose.

5. Concepts of the bacterial cell wall are discussed in the light of these findings.

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REFERENCES

- Berenblum, I. & Chain, E. (1938). *Biochem. J.* **32**, 295.
Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
Cooper, P. D., Rowley, D. & Dawson, I. M. (1949). *Nature, Lond.*, **164**, 842.
Curran, H. R. & Evans, F. R. (1942). *J. Bact.* **43**, 125.
Dawson, I. D. (1949). In Miles, A. A. & Pirie, N. W., *The Nature of the Bacterial Cell Surface*. Oxford: Blackwell Scientific Publications.
Dische, Z. (1930). *Mikrochemie*, **8**, 4.
Fernell, R. W. & King, H. K. (1953). *Analyst*, **78**, 80.
Freeman, G. G., Challinor, S. W. & Wilson, J. (1940). *Biochem. J.* **34**, 307.
Henry, H. & Stacey, M. (1946). *Proc. Roy. Soc. B*, **133**, 391.
Holdsworth, E. S. (1952). *Biochim. biophys. Acta*, **9**, 19.
King, H. K. & Alexander, H. (1948). *J. gen. Microbiol.* **2**, 315.
King, H. K. & Stein, J. (1950). *J. gen. Microbiol.* **4**, 48.
Malmgren, B. & Heden, C. (1947). *Acta path. microbiol. scand.* **24**, 417.
Markham, R. (1942). *Biochem. J.* **36**, 790.
Mitchell, P. (1950). *J. gen. Microbiol.* **4**, 399.
Mitchell, P. & Moyle, J. (1951). *J. gen. Microbiol.* **5**, 981.
Morgan, W. T. J. & Partridge, S. M. (1940). *Biochem. J.* **34**, 169.
Northcote, D. H. & Horne, R. W. (1952). *Biochem. J.* **51**, 232.
Salton, M. J. R. (1952a). *Biochim. biophys. Acta*, **8**, 510.
Salton, M. J. R. (1952b). *Biochim. biophys. Acta*, **9**, 334.
Salton, M. J. R. & Horne, R. W. (1951a). *Biochim. biophys. Acta*, **7**, 19.
Salton, M. J. R. & Horne, R. W. (1951b). *Biochim. biophys. Acta*, **7**, 177.
Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.

The Mechanism of Inhibition of Cholinesterases by Organophosphorus Compounds

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In a previous paper it was concluded that organophosphorus compounds inhibit cholinesterase by phosphorylating the enzyme and a chemical mechanism was suggested for both inhibition and reversal of inhibition (Aldridge, 1953a). A general formula of organophosphorus inhibitors may be represented as $(R)_3(X)PO$ where X is the group labile to hydrolysis and R is usually an alkoxy group. According to the phosphorylation theory, provided the R group is maintained constant, even though the X group is varied, the same inhibited enzyme must result. Wilson (1952) has shown that the inhibition

of electric-eel cholinesterase after both tetraethyl pyrophosphate and diethyl phosphorofluoridate reverses at the same extremely slow rate and is also increased to the same degree by the presence of high concentrations of choline.

True cholinesterase activity returns quickly after inhibition by dimethyl *p*-nitrophenyl phosphate (Aldridge, 1953a) and the inhibition of rat pseudo cholinesterase by diethyl *p*-nitrophenyl phosphate has also been shown to reverse readily (Davison, 1953). Thus after inhibition of these enzymes by a series of inhibitors the identity or otherwise of the