BRITISH PHARMACEUTICAL CONFERENCE

NINETY-NINTH ANNUAL MEETING, LIVERPOOL, 1962

REPORT OF PROCEEDINGS

OFFICERS:

President:

Miss M. A. BURR, M.P.S., Nottingham

Chairman:

J. C. HANBURY, M.A., B.Pharm., F.P.S., F.R.I.C.

Vice-Chairmen:

R. R. BENNETT, B.Sc., F.P.S., F.R.I.C., Eastbourne.
H. DEANE, B.Sc., F.P.S., F.R.I.C., Sudbury.
H. HUMPHREYS JONES, F.P.S., F.R.I.C., Liverpool.
T. E. WALLIS, D.Sc., F.P.S., F.R.I.C., F.L.S., London.
H. BRINDLE, M.Sc., F.P.S., F.R.I.C., Altrincham.
N. Evers, B.Sc., Ph.D., F.R.I.C., Ware.
A. D. POWELL, M.P.S., F.R.I.C., Nottingham.
H. BERRY, B.Sc., Dip.Bact. (Lond.), F.P.S., F.R.I.C., Eastbourne.
H. B. MACKIE, B.Pharm., F.P.S., Brighton.
G. R. BOYES, L.M.S.S.A., B.Sc., F.P.S., F.R.I.C., London.
H. DAVIS, C.B.E., B.Sc., Ph.D., F.P.S., F.R.I.C., London.
J. P. TODD, Ph.D., F.P.S., F.R.I.C., Glasgow.
K. BULLOCK, M.Sc., Ph.D., F.P.S., F.R.I.C., Manchester.
F. HARTLEY, B.Sc., Ph.D., F.P.S., F.R.I.C., London.
G. E. FOSTER, B.Sc., Ph.D., F.R.I.C., Dartford.
H. TREVES BROWN, B.Sc., F.P.S., London.
W. H. LINNELL, D.Sc., Ph.D., F.P.S., F.R.I.C., London.
D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., Nottingham

Honorary Treasurer:

H. G. ROLFE, B.Sc., F.P.S., F.R.I.C., London.

Honorary General Secretaries:

E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., London.
D. TRAIN, M.C., Ph.D., F.P.S., F.R.I.C., A.M.I.Chem.E., London

Other Members of the Executive Committee:

THE CHAIRMAN of the Executive of the Scottish Department of the Pharmaceutical Society of Great Britain (*ex officio*).
THE PRESIDENT of the Pharmaceutical Society of Ireland (*ex officio*).
THE PRESIDENT of the Pharmaceutical Society of Northern Ireland (*ex officio*).
THE EDITOR of the *Journal of Pharmacy and Pharmacology* (*ex officio*).
THE CHAIRMAN and Honorary Secretary of the Local Committee (*ex officio*).

A. H. BECKETT, D.Sc., Ph.D., F.P.S., F.R.I.C., London.
K. R. CAPPER, Ph.D., B.Pharm., F.P.S., D.I.C., London.
*H. S. GRAINGER, F.P.S., London.
*C. W. MAPLETHORPE, M.Sc., F.P.S., F.R.I.C., M.I.Chem. E., Ware.
*T. REID, M.P.S., Haslemere.
E. SHOTTON, B.Sc., Ph.D., F.P.S., F.R.I.C. London.
G. F. SOMERS, B.Sc., Ph.D., F.P.S., M.I.Biol., Liverpool.
R. E. STUCKEY, D.Sc., Ph.D., F.P.S., F.R.I.C., London.
W. T. Wing, F.P.S., D.B.A., Newcastle.

* Members nominated by the Council of the Pharmaceutical Society of Great Britain.

REPORT OF PROCEEDINGS

PROCEEDINGS OF CONFERENCE

LIVERPOOL, 1962

THE OPENING SESSION

The opening session of the Conference was held in the Philharmonic Hall on Monday, September 10, with Miss M. A. Burr, President of the Conference (President of the Pharmaceutical Society) in the Chair. On the platform were the Chairman of the Conference (Mr. J. C. Hanbury), the Lord Mayor of Liverpool (Alderman D. J. Lewis) and the Mayors of Wallasey, Birkenhead and Southport, the Pro-Vice-Chancellor, University of Liverpool (Professor F. E. Hyde), Lord Cohen of Birkenhead, the Principal of the City of Liverpool College of Technology (Mr. S. A. J. Parsons), the Chairman of the Local Committee (Professor A. Wilson), the Honorary General Secretaries, the Officers of the Local Committee, together with members of the Conference Executive.

The President introduced the Lord Mayor of Liverpool, who welcomed the Conference to the City. The President thanked the Lord Mayor on behalf of the Conference for his welcome.

The President then handed over further conduct of the Conference to the Chairman (Mr. J. C. Hanbury), who delivered his address entitled "The Role of Pharmaceutical Sciences in Medicine," which is printed in the *Pharmaceutical Journal*, 1962, **189**, 243–246.

On the proposition of Mr. C. W. Robinson, the Conference accorded a vote of thanks to the Chairman for his address.

UNIVERSITY RECEPTION

A Reception by Liverpool University in honour of the Conference was held at Tate Hall on the evening of Monday, September 10. The guests were welcomed by Professor L. Rosenhead (Pro-Vice-Chancellor).

CIVIC RECEPTION

On the evening of Wednesday, September 12, a Civic Reception was given at the Walker Art Gallery. The guests were received by the Lord Mayor (Alderman D. J. Lewis) and the Lady Mayoress, and by the Chairman of the Liverpool Branch of the Pharmaceutical Society (Mr. A. L. Saul) and Mrs. Saul.

THE SCIENCE SESSIONS

Meetings were held on Monday, Tuesday and Friday, September 10, 11 and 14 at the Donnan Laboratories, the Chairman and immediate past Chairman presiding. During the sessions the following 25 papers were presented.

1. An Investigation of the Metabolism of Neostigmine in Patients with Myasthenia Gravis. By Carol A. Scott, B.Sc., Ph.D., P. T. Nowell, B.Sc., Ph.D. and A. Wilson, M.D., Ph.D., F.R.F.P.S.
2. Some Effects of a Hemicholinium Compound (HC–3) on Neuromuscular Transmission in the Cat. By E. R. Evans, M.D. and H. Wilson, M.D., Ph.D.
3. Myasthenic-like Features of the Neuromuscular Transmission Failure Produced by Triethylcholine. By W. C. Bowman, B.Pharm., Ph.D., M.P.S., B. A. Hemsworth, B.Pharm., F.P.S. and M. J. Rand, M.Sc., Ph.D.
4. The Response of the Pig Uterus to Oxytocin at Different Stages in the Oestrus Cycle. By A. Knifton, B.V.Sc., B.Sc., M.R.C.V.S.
5. An Apparatus for Testing Anticonvulsant Drugs by Electroshock Seizures in Mice. By C. H. Cashin, B.Pharm., M.P.S. and H. Jackson, B.Sc. (Pharm.), M.P.S.
6. Local Anaesthetic Activity in Diethylaminoacetyl Derivatives of Substituted Benzylamines. By R. F. Collins, B.Sc., Ph.D., A.R.I.C. and B. J. Large.
7. The Determination of Ergotamine in Preparations Containing Ergotamine Tartrate and Cyclizine Hydrochloride. By A. C. Caws, B.Sc., A.R.I.C. and B. E. Lawrence, B.Sc., A.R.C.S.
8. The Determination of Calcium in Heavy Magnesium Carbonate using Glyoxal bis(2-hydroxyanil). By M. A. Leonard, B.Sc., Ph.D., A.R.I.C.
9. The Analysis of Poldine Methosulphate by Infra-red Spectroscopy. By H. D. C. Rapson, B.Sc., Ph.D., D.I.C., A.R.I.C., K. W. Austin and E. A. Cutmore, B.Sc., M.Sc.
10. The Colorimetric Determination of Phenolphthalein. By J. Allen, F.R.I.C., (Miss) B. Gartside, B.Pharm., M.P.S. and C. A. Johnson, B.Pharm., B.Sc., F.P.S., F.R.I.C.

11. The use of Tetraphenylboron for the Determination and Characterisation of Organic Bases in Pharmaceutical Preparations. By C. A. Johnson, B.Pharm., B.Sc., F.P.S., F.R.I.C. and R. E. King, A.R.I.C.
12. Water for Injection by Ion-exchange. By A. M. Cook, B.Pharm., Ph.D., Dip.Bact., F.P.S., F.R.I.C. and L. Saunders, D.Sc., Ph.D., F.R.I.C.
13. Some Physical Properties of Interfacial Films of Potassium Arabate. By K. Wibberley, B.Pharm., M.P.S., A.R.I.C.
14. A Note on the Stability of Solutions of Isoprenaline. By G. B. West, B.Pharm., D.Sc., Ph.D., F.P.S. and T. D. Whittet, B.Sc., Ph.D., F.P.S., F.R.I.C.
15. The Assay of Protamine Sulphate for its Capacity to Neutralise Heparin. By V. J. Birkinshaw, F.P.S. and K. L. Smith, M.P.S.
16. The Oxidation of Emulsified and Solubilised Benzaldehyde. By J. E. Carless, B.Pharm., M.Sc., Ph.D., F.P.S. and J. Swarbrick, B.Pharm., M.P.S.
17. Surface-activity of a Series of Synthetic Non-ionic Detergents. By P. H. Elworthy, B.Pharm., Ph.D., M.P.S., A.R.I.C. and C. B. Macfarlane, B.Sc., M.P.S.
18. The Controlled Potential Reduction of Crystal Violet and Brilliant Green at the Stirred Mercury Cathode. By C. G. Butler, B.Pharm., Ph.D., M.P.S., F.R.I.C. and (Mrs.) F. P. Martin, M.P.S.
19. Studies on *Datura leichhardtii* Muell. ex Benth. Part II. Alkaloidal Constituents. By W. C. Evans, B.Pharm., B.Sc., Ph.D., F.P.S. and N. A. Stevenson, M.Pharm., B.Sc., M.P.S.
20. The Particle Size Distribution of Marble on Wet Ball Milling. Effect of the Solid: Liquid Ratio. By M. I. Barnett, B.Pharm., M.P.S. and K. C. James, M.Pharm., F.P.S., A.R.I.C.
21. Determination of Trichloroethyl Phosphate in Pharmaceutical Preparations. By P. F. G. Boon, B.Sc., A.R.I.C.
22. The Effect of a Sulphated Polysaccharide on the Acidity and Volume of Histamine-stimulated Gastric Secretion in the Guinea-pig. By W. Anderson, B.Sc., Ph.D., F.P.S., R. Marcus, M.D., Ch.M., F.R.C.S. and J. Watt, M.D.
23. The Polarographic Assay of Streptomycin. By R. Goodey, F.R.I.C., T. E. Couling, F.R.I.C. and (Miss) J. E. Hart, B.Sc.
24. Studies on the Postirradiation Oxygen Effect in Bacterial Spores. By A. Tallentire, M.Sc., Ph.D. and N. A. Dickinson, B.Pharm.
25. The Effect of Age on the Viability of *Penicillium notatum* Spores in Water and Solutions of Phenol. By N. M. Chauhan, B.Pharm., M.P.S. and V. Walters B.Pharm., Ph.D., F.P.S.

THE CONFERENCE LECTURE

A lecture on "A Study of Inherited Variability in the Response to Drugs" was given on Wednesday, Setember 12, by Dr. C. A. Clarke. The Chairman presided. The lecture is printed in the *Journal of Pharmacy and Pharmacology*, **14**, 20 *T*–30 *T*.

THE SYMPOSIUM SESSION

A symposium on "Drug Addiction" was held on Thursday, September 13. The Chairman presided. The introductory papers were by Prof. A. D. Macdonald, Dr. J. M. Johnston and Mr. B. J. Thomas. The meeting is reported in the *Journal of Pharmacy and Pharmacology*, **14**, 9 *T*–19 *T*.

PROFESSIONAL SESSIONS

With the President of the Conference, Miss M. A. Burr, in the Chair, professional sessions were held on the mornings of Tuesday, September 11, when Mr. F. W. Adams made the opening remarks to "Further Discussion of the Report of the Committee on the General Practice of Pharmacy," and Friday, September 14, when Mr. N. Herdman read an introductory paper to the subject "The Employee Pharmacist." Full reports of the papers and discussions were published in the *Pharmaceutical Journal*, 1962, **189**, 271–276; 309–318.

THE CLOSING SESSION

The closing session of the Conference was held on Friday, September 14, in the Philharmonic Hall, the Chairman presiding.

Vote of Thanks to Local Committee

The Chairman called on Mr. H. H. Campbell to propose a vote of thanks to the Local Committee. This was seconded by Dr. R. F. White. Prof. A. Wilson (Chairman of the Local Committee) replied to the vote of thanks. The Chairman then

presented to the Liverpool and District Branch an inscribed gavel provided by the Bell and Hills Fund. Mr. A. L. Saul (Chairman of the Branch) accepted and acknowledged the gift on behalf of the Branch.

Annual Report

Dr. E. F. Hersant presented the Annual Report of the Executive Committee.

Your Executive has pleasure in presenting the ninety-ninth Annual Report.

Membership.—In addition to Members, Honorary Members and Students of the Pharmaceutical Society, together with the Members of the Pharmaceutical Society of Northern Ireland, the Conference includes seventy-one members elected by the Executive who are not members of these Societies.

Reports on 1961 Meeting.—The report of the meeting of the Conference at Portsmouth 1961 together with the science papers and discussions, and the Conference Lecture were published as a supplement to the 13th Volume of the *Journal of Pharmacy and Pharmacology*. The papers and discussions at the Professional Sessions were published in the *Pharmaceutical Journal*, Series IV, Volume 133.

Conference Papers, 1962.—Thirty-one research papers were submitted. Twelve full papers and thirteen short communications were accepted for presentation. The Executive thanks the authors of these papers and also the authors of the papers presented to the Symposium and the Professional Sessions for their contributions. The Executive is grateful to the Editor of the *Journal of Pharmacy and Pharmacology* and to the Editor of the *Pharmaceutical Journal* for making galley proofs of the papers available before this meeting.

Conference Lecture, 1962.—Following the precedent set in 1961, a Conference Lecture was delivered by Dr. C. A. Clarke of the Department of Medicine, University of Liverpool, on "Pharmacogenetics—A Study of Inherited Variability in the Response to Drugs." The Executive wish to record their thanks to him for his contribution to the Conference.

Journal of Pharmacy and Pharmacology.—The Executive has been represented on the Editorial Board by the Chairman (Mr. J. C. Hanbury), the immediate Past-Chairman (Dr. D. C. Garratt) and the Senior Honorary General Secretary.

Future Meetings.—An invitation will be presented at this meeting for the Conference to meet in London during the week commencing September 2, 1963. As reported last year, an invitation to meet in Edinburgh during the week commencing September 14, 1964 has been provisionally accepted, and your Executive has since provisionally accepted an invitation to hold the Conference in Cardiff during the week commencing September 6, 1965. Several Branches of the Society have made preliminary enquiries regarding the possibility of entertaining the Conference in future years and the Executive is grateful for all these offers of hospitality.

Constitution and Rules. The Sub-Committee appointed to review the Constitution of the Executive and the Rules of the Conference submitted proposals for new Rules which were accepted by your Executive. These proposed Rules have been published in the pharmaceutical press and distributed to members attending the Conference. They will be submitted for your approval later at this meeting.

Officers and Executive of the Conference.—Your Executive has nominated the following Officers and Members of the Executive for 1962–63:

Chairman: H. G. Rolfe; *Vice-Chairmen:* R. R. Bennett, H. Deane, H. Humphreys Jones, T. E. Wallis, H. Brindle, Norman Evers, A. D. Powell, H. Berry, H. B. Mackie, G. R. Boyes, H. Davis, J. P. Todd, K. Bullock, Frank Hartley, G. E. Foster, H. Treves Brown, W. H. Linnell, D. C. Garratt and J. C. Hanbury; *Honorary Treasurer:* J. M. Rowson; *Honorary General Secretaries:* E. F. Hersant and D. Train. Other members of the Executive: A. H. Beckett, K. R. Capper, D. W. Hudson, E. Shotton, R. E. Stuckey and G. Sykes.

The above persons together with the President of the Conference (the President of the Pharmaceutical Society of Great Britain *ex officio*), the three persons nominated by the Council of the Pharmaceutical Society of Great Britain, namely the persons for the time being holding the office of Vice-President, immediate past President and Chairman of the Organisation Committee, together with the

following *ex officio:* The Chairman of the Executive of the Scottish Department, the President of the Pharmaceutical Society of Ireland, the President of the Pharmaceutical Society of Northern Ireland, the Editor of the *Journal of Pharmacy and Pharmacology*, the Chairman of the Local Committee and the Honorary Local Secretary, will form the Executive for 1962–63.

ACKNOWLEDGMENTS.—The Executive wishes to record thanks to the Chairman, Officers and Members of the Liverpool Local Committee for their work in making the local arrangements, and to the Council of the Pharmaceutical Society of Great Britain for the continued provision of secretarial and other facilities.

Mr. J. H. Oakley proposed the acceptance of the report and the election of officers of the Conference for the ensuing year. Miss G. M. Watson seconded.

Mr. H. G. Rolfe thanked the Conference on behalf of the newly-elected officers.

TREASURER'S REPORT

During the financial year ending December 31, 1961, the Local Committee Fund of £250 having been repaid by the Portsmouth Local Committee, was loaned to the Liverpool Local Committee in respect of the 1962 Conference.

The subscriptions of the elected members, including a composition fee from the Pharmaceutical Society of Northern Ireland, amounted to £144 and were credited to the account of the *Journal of Pharmacy and Pharmacology*.

H. G. ROLFE,
Honorary Treasurer.

BRITISH PHARMACEUTICAL CONFERENCE ACCOUNT

INCOME AND EXPENDITURE ACCOUNT, 1961

	£	s.	d.	*Income*	£	s.	d.
Gavel—memento to Portsmouth Branch	9	6	6	Interest on 2½% Consols	40	5	0
Replica of Chairman's Badge, engraving, etc.	8	2	0	Interest on 3% Savings Bonds	6	0	0
Ribbon for Chairman's Badge	–	–	–	Interest on 3% Exchequer Stock	15	0	0
Engraving Sports Trophies	1	8	6	Interest on Post Office Bank Account	–	–	–
Secretaries' Expenses	4	0	0	Interest on Bank Deposit Account	4	8	5
Expenses of Speakers	32	6	3	Donation from Pharmaceutical Society of Northern Ireland	25	0	0
Honorarium to Conference Lecturer	26	5	0	Donation from Pharmaceutical Society of Ireland	25	0	0
Income Tax	24	17	11				
Cheque Book		4	0				
Surplus carried to Accumulated Fund	9	3	3				
	£115	13	5		£115	13	5

BALANCE SHEET AT DECEMBER 31, 1961

Liabilities	£	s.	d.	*Assets*	£	s.	d.
Accumulated Fund, as at 31.12.60	2,059	17	1	Investments at cost (*a*) £1,610 2½% Consols (Donation by the late Alderman Clayton of Birmingham)	1,250	0	0
Add: Surplus 1961	9	3	3	(*b*) £200 3% Savings Bonds 1960–70	200	0	0
	2,069	0	4	(*c*) £500 3% Exchequer Stock 1962–63	473	4	10
Creditor		10	6	(Total market value at December 31, 1961: £1,253, 1960: £1,343.)			
Local Committee Fund:—					1923	4	10
Donation from London Committee, 1953	250	0	0	Stock of Replicas (4) of Chairman's Badge	29	8	0
				Loan to Liverpool Local Committee	250	0	0
Audited and found correct				Cash at Bank—			
T. HESELTINE				Deposit Account	101	4	4
T. C. DENSTON				Current Account	15	13	8
May 2, 1962							
	£2,319	10	10		£2,319	10	10

REVISED CONSTITUTION AND RULES

The Chairman submitted to the meeting the Revised Constitution and Rules which had been previously published in the *Pharmaceutical Journal*, 1962, **189**, 156, and circulated to members of the Conference. After discussion, they were approved to come into force from the date of the London Conference, 1963.

REPORT OF PROCEEDINGS

BRITISH PHARMACEUTICAL CONFERENCE

INAUGURAL MEETING HELD AT NEWCASTLE UPON TYNE IN 1863

Years	*Places of Meeting*	*Presidents*	*Local Secretaries*
1864	BATH	H. DEANE, F.L.S.	J. C. POOLEY
1865	BIRMINGHAM ..	H. DEANE, F.L.S.	W. SOUTHALL, JUN.
1866	NOTTINGHAM ..	PROF. R. BENTLEY, F.L.S.	J. H. ATHERTON
1867	DUNDEE ..	PROF. R. BENTLEY, F.L.S.	J. HODGE
1868	NORWICH ..	D. HANBURY, F.R.S.	F. SUTTON
1869	EXETER ..	D. HANBURY, F.R.S.	M. HUSBAND
1870	LIVERPOOL ..	W. W. STODDART	E. DAVIES J. DUTTON (Birkenhead)
1871	EDINBURGH ..	W. W. STODDART	J. MACKAY
1872	BRIGHTON ..	H. B. BRADY, F.R.S.	T. GLAISYER
1873	BRADFORD ..	H. B. BRADY, F.R.S.	R. PARKINSON, PH.D.
1874	LONDON ..	T. B. GROVES	M. CARTEIGHE
1875	BRISTOL ..	T. B. GROVES	J. PITMAN
1876	GLASGOW ..	PROF. T. REDWOOD	A. KINNINMONT
1877	PLYMOUTH ..	PROF. T. REDWOOD	R. J. CLARK
1878	DUBLIN ..	G. F. SCHACHT	W. HAYES
1879	SHEFFIELD ..	G. F. SCHACHT	H. MALEHAM
1880	SWANSEA ..	W. SOUTHALL, F.L.S.	J. HUGHES
1881	YORK	R. REYNOLDS	J. OWRAY
1882	SOUTHAMPTON	PROF. J. ATTFIELD, F.R.S.	O. R. DAWSON
1883	SOUTHPORT ..	PROF. J. ATTFIELD, F.R.S.	W. ASHTON
1884	HASTINGS ..	J. WILLIAMS	F. ROSSITER
1885	ABERDEEN ..	J. B. STEPHENSON	A. STRACHAN
1886	BIRMINGHAM ..	T. GREENISH	C. THOMPSON
1887	MANCHESTER ..	S. R. ATKINS, J.P.	F. B. BENGER
1888	BATH	F. B. BENGER	H. HUTTON
1889	NEWCASTLE UPON TYNE	C. UMNEY, F.I.C.	T. M. CLAGUE
1890	LEEDS ..	C. UMNEY, F.I.C.	F. W. BRANSON
1891	CARDIFF ..	W. MARTINDALE	A. COLEMAN
1892	EDINBURGH ..	E. C. C. STANFORD	P. BOA
1893	NOTTINGHAM ..	O. CORDER	C. A. BOLTON
1894	OXFORD ..	N. H. MARTIN, F.L.S., F.R.M.S.	H. MATTHEWS
1895	BOURNEMOUTH	N. H. MARTIN, F.L.S., F.R.M.S.	S. HARDWICK
1896	LIVERPOOL ..	W. MARTINDALE	T. H. WARDLEWORTH H. O. DUTTON (Birkenhead)
1897	GLASGOW ..	C. SYMES, PH.D.	J. A. RUSSELL
1898	BELFAST ..	C. SYMES, PH.D.	R. W. McKNIGHT W. J. RANKIN
1899	PLYMOUTH ..	J. C. C. PAYNE, J.P.	J. DAVY TURNEY
1900	LONDON ..	E. M. HOLMES, F.L.S.	W. WARREN H. CRACKNELL
1901	DUBLIN ..	G. C. DRUCE, M.A., F.L.S.	J. I. BERNARD
1902	DUNDEE ..	G. C. DRUCE, M.A., F.L.S.	W. CUMMINGS
1903	BRISTOL ..	T. H. W. IDRIS, M.P.	H. E. BOORNE
1904	SHEFFIELD ..	T. H. W. IDRIS, M.P.	H. ANTCLIFFE
1905	BRIGHTON ..	W. A. H. NAYLOR, F.I.C.	W. W. SAVAGE C. G. YATES
1906	BIRMINGHAM ..	W. A. H. NAYLOR, F.I.C.	C. THOMPSON
1907	MANCHESTER ..	T. TYRER, F.I.C.	W. KIRBY
1908	ABERDEEN ..	R. WRIGHT	W. F. HAY
1909	NEWCASTLE UPON TYNE	J. G. TOCHER, B.Sc.	T. M. CLAGUE H. W. NOBLE
1910	CAMBRIDGE ..	F. RANSOM	A. A. DECK T. J. MALLETT
1911	PORTSMOUTH ..	W. F. WELLS	T. O. BARLOW T. POSTLETHWAIT
1912	EDINBURGH ..	SIR EDWARD EVANS, J.P.	T. STEPHENSON
1913	LONDON ..	J. C. UMNEY	W. J. U. WOOLCOCK
1914	CHESTER ..	E. H. FARR	R. C. OWEN, B.Sc.
1915	LONDON ..	E. SAVILLE PECK, M.A.	———
1916	LONDON ..	D. HOOPER, LL.D., F.R.I.C.	———
1917	LONDON ..	C. A. HILL, B.Sc., F.R.I.C.	———
1918	LONDON ..	C. A. HILL, B.Sc., F.R.I.C.	———
1919	LONDON ..	W. KIRBY, M.Sc.	———
1920	LIVERPOOL ..	C. A. HILL, B.Sc., F.R.I.C.	H. HUMPHREYS JONES, F.R.I.C.
1921	SCARBOROUGH	E. SAVILLE PECK, M.A.	E. R. CROSS
1922	NOTTINGHAM ..	PROF. H. G. GREENISH, D. ès. Sc., F.I.C.	E. C. CARR

Years	*Places of Meeting*	*Chairmen*	*Local Secretaries*
1923	LONDON ..	F. W. GAMBLE	W. J. U. WOOLCOCK, C.B.E.
1924	BATH	E. WHITE, B.Sc., F.I.C.	P. J. THOMPSON W. H. HALLETT
1925	GLASGOW ..	E. WHITE, B.Sc., F.I.C.	P. M. DUFF
1926	LEICESTER ..	D. LLOYD HOWARD, J.P.	J. BARKER
1927	BRIGHTON ..	D. LLOYD HOWARD, J.P.	F. W. BURGESS
1928	CHELTENHAM ..	R. R. BENNETT, B.Sc., F.R.I.C.	P. JAMES
1929	DUBLIN ..	R. R. BENNETT, B.Sc., F.R.I.C.	V. E. HANNA

Years	*Places of Meeting*	*Chairmen*	*Local Secretaries*
1930	CARDIFF ..	J. T. HUMPHREY	J. MURRAY
1931	MANCHESTER ..	J. H. FRANKLIN	R. G. EDWARDS
1932	ABERDEEN ..	H. SKINNER	H. M. DUGAN
1933	LONDON ..	C. H. HAMPSHIRE, C.M.G., M.B., B.S., B.Sc., F.R.I.C.	H. N. LINSTEAD
1934	LEEDS ..	C. H. HAMPSHIRE, C.M.G., M.B., B.S., B.Sc., F.R.I.C.	G. C. CRUMMACK J. F. SIMON
1935	BELFAST ..	F. W. CROSSLEY-HOLLAND, L.M.S.S.A.	D. L. KIRKPATRICK
1936	BOURNEMOUTH	H. DEANE, B.Sc., F.R.I.C.	V. J. SCAMPTON
1937	LIVERPOOL ..	T. E. LESCHER, O.B.E.	W. E. HUMPHREYS
1938	EDINBURGH ..	J. RUTHERFORD HILL, O.B.E.	C. G. DRUMMOND
1939	BIRMINGHAM ..	J. RUTHERFORD HILL, O.B.E.	D. J. RUSTON
1940	LONDON ..	H. HUMPHREYS JONES, F.R.I.C.	——
1941	LONDON ..	A. R. MELHUISH	——
1942	LONDON ..	T. E. WALLIS, D.Sc., F.R.I.C., F.L.S.	——
1943	LONDON ..	T. E. WALLIS, D.Sc., F.R.I.C., F.L.S.	——
1944	LONDON ..	H. BRINDLE, B.Sc., F.R.I.C.	——
1945	LONDON ..	H. BRINDLE, B.Sc., F.R.I.C.	——
1946	LONDON ..	B. A. BULL, A.R.I.C.	——
1947	TORQUAY ..	B. A. BULL, A.R.I.C.	T. D. EVANS
1948	BRIGHTON ..	N. EVERS, B.Sc., PH.D., F.R.I.C.	A. WILSON
1949	BLACKPOOL ..	N. EVERS, B.Sc., PH.D., F.R.I.C.	R. VARLEY T. A. DURKIN
1950	GLASGOW ..	A. D. POWELL, F.R.I.C.	A. OFFICER
1951	HARROGATE ..	H. BERRY, B.Sc., Dip. Bact. (Lond.), F.R.I.C.	R. W. JACKSON
1952	NOTTINGHAM ..	H. B. MACKIE, B.Pharm.	W. E. NEWBOLD Miss G. M. WATSON
1953	LONDON ..	G. R. BOYES, L.M.S.S.A., B.Sc., F.R.I.C.	J. M. ROWSON
1954	OXFORD ..	H. DAVIS, C.B.E., B.Sc., PH.D., F.R.I.C.	T. R. HARDY
1955	ABERDEEN ..	J. P. TODD, PH.D., F.R.I.C.	D. L. DICKIE
1956	DUBLIN ..	K. BULLOCK, M.Sc., PH.D., F.R.I.C.	D. J. KENNELLY
1957	BRISTOL ..	F. HARTLEY, B.Sc., PH.D., F.R.I.C.	E. GEORGE
1958	LLANDUDNO ..	G. E. FOSTER, B.Sc., PH.D., F.R.I.C.	M. H. THOMAS
1959	BOURNEMOUTH	H. TREVES BROWN, B.Sc., F.P.S.	D. F. SMITH
1960	NEWCASTLE UPON TYNE	W. H. LINNELL, D.Sc., PH.D., F.P.S., F.R.I.C.	A. McGUCKIN
1961	PORTSMOUTH ..	D. C. GARRATT, D.Sc., PH.D., F.R.I.C.	N. L. BANKS
1962	LIVERPOOL ..	J. C. HANBURY, M.A., B.PHARM., F.P.S., F.R.I.C.	D. L. REES

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1958 to D. TRAIN, M.C., B.Pharm., B.Sc., PH.D., F.R.I.C., A.M.I.Chem.E.

Closing Session (continued)

PLACE OF MEETING FOR 1963

Mr. A. Aldington on behalf of the Metropolitan Branches of the Society, extended an invitation to hold the Conference in London in 1963. Mr. J. R. Phillips proposed that the invitation be accepted, and Miss A. G. Esmonde seconded. The vote was put to the meeting and unanimously carried.

VOTE OF THANKS TO CHAIRMAN

Mr. G. Bryan proposed a vote of thanks to the Chairman.

Mr. D. G. Beckwith seconded. The vote was put to the meeting by the President and carried with acclamation.

Mr. Hanbury briefly responded.

REPORT OF PROCEEDINGS

BRITISH PHARMACEUTICAL CONFERENCE

CONSTITUTION AND RULES

1. The British Pharmaceutical Conference shall meet annually for the discussion of subjects relative to the science of pharmacy, for the promotion of friendly reunion amongst pharmacists and those interested in pharmacy, and generally for the furtherance of the objects sought to be obtained under the Royal Charter of Incorporation granted to the Pharmaceutical Society of Great Britain and the several Pharmacy Acts.

2. The Conference shall consist of:

(*a*) members honorary members and student-associates of the Pharmaceutical Society of Great Britain;

(*b*) members of the other Pharmaceutical Societies within the British Commonwealth of Nations which desire to be associated with the work of the Conference and have made an agreement with the British Pharmaceutical Conference whereby an annual subscription shall be paid by their Society in lieu of individual subscriptions from members; and

(*c*) persons at home and abroad interested in the advancement of Pharmacy who, not being qualified for membership of the Pharmaceutical Society of Great Britain or one of the other Societies associated with the work of the Conference, have been nominated in writing by a member of the Conference, and elected by the Executive.

3. The Officers of the Conference, who shall collectively constitute the Executive Committee, shall be: A Chairman, Vice-Chairmen, one Honorary Treasurer, two Honorary General Secretaries, together with three members of the Council of the Pharmaceutical Society of Great Britain, and six other members of the Conference. Of the six other members nominated annually by the outgoing Executive the two members who have had the longest period of continuous service shall be ineligible for re-nomination for one year. The President of the Pharmaceutical Society of Great Britain shall be *ex officio* a member of the Executive Committee and President of the Conference. The Chairman of the Executive of the Scottish Department of the Pharmaceutical Society of Great Britain, the President of the Pharmaceutical Society of Ireland, the President of the Pharmaceutical Society of Northern Ireland, the President of any other Pharmaceutical Society the members of which are members of the Conference, the Editor of the *Journal of Pharmacy and Pharmacology*, the Chairman of the Local Committee, and the Honorary Local Secretary shall be *ex officio* members of the Executive Committee.

4. The Officers of the Conference shall be selected at each annual meeting by those present. Nominations for membership of the Executive Committee may be made at the annual meeting by the outgoing Executive and shall include nominations which have been received by the Honorary Secretaries in writing at least twenty-eight days before the annual meeting.

5. The Chairman of the Conference shall give the inaugural address, preside over the meetings for the reading of scientific papers and take the chair at the meetings of the Executive Committee.

6. Members, honorary members and student-associates of the Pharmaceutical Society of Great Britain shall not be required to pay a separate subscription to the British Pharmaceutical Conference; they shall be entitled, on application, to receive the issues of the *Journal of Pharmacy and Pharmacology*, as published, on preferential terms.

Members of any other Pharmaceutical Society, who are members of the Conference by virtue of the arrangement between their Society and the British Pharmaceutical Conference, shall not be required to pay a separate subscription to the British Pharmaceutical Conference; they shall be entitled, on application to receive the *Journal of Pharmacy and Pharmacology*, as published, on preferential terms.

Other members elected by the Executive shall pay a subscription of 55s. annually, which shall entitle them, on application, to receive the *Journal of Pharmacy and Pharmacology*, as published. Subscriptions shall become due on January 1, and membership shall cease if subscriptions are not paid by June 1.

BRITISH PHARMACEUTICAL CONFERENCE
Liverpool, 1962

Chairman: J. C. Hanbury*

SYMPOSIUM
DRUG ADDICTION
FIRST INTRODUCTORY ADDRESS

BY A. D. MACDONALD, M.D., M.A., M.Sc.

Professor of Pharmacology, The University, Manchester

THE use or misuse of drugs leading to habit and addiction is an old problem to the doctor and the pharmacist. In Britain it is not a very large or serious one and certainly not to be compared in size to the problems arising from addiction to alcohol. But drug fashions and habits change and a one-time serious danger disappears or is dwarfed by a new difficulty. In particular, the development of synthetic substitutes for morphine in the last 25 years has modified the opium problem, while the increase in the use of barbiturates and the so-called tranquillisers is disquieting.

In 1958 an Interdepartmental Committee on Drug Addiction was set up by the Minister of Health and the Secretary of State for Scotland "to review the advice given by the Departmental Committee on Morphine and Heroin Addiction (the Rolleston Committee) in 1926, to consider whether any revised advice should also cover other drugs liable to produce addiction or to be habit-forming; to consider whether there is a medical need to provide special, including institutional, treatment outside the resources already available, for persons addicted to drugs; and to make recommendations, including proposals for any administrative measures that seem expedient." I had the honour of serving upon that Committee and have for some years had close contact with the Society for the Study of Addiction and as a member till recently of the Council of the P.S.G.B. have some experience of how pharmacists are involved in the problems that addiction to drugs can raise.

Before these problems can be discussed it is desirable that definitions be made which are generally, if not always, accepted. The Interdepartmental Committee in its report (1961) has slightly modified the WHO definitions of Addiction and Habituation, as follows.

"*Drug Addiction* is a state of periodic or chronic intoxication produced by the repeated consumption of a drug (natural or synthetic); its characteristics include:

1. An overpowering desire or need (compulsion) to continue taking the drug and to obtain it by any means.

* The Chairman's Address entitled "The Role of the Pharmaceutical Sciences in Medicine" is published in the *Pharmaceutical Journal*, 1962, **189**, 243–246.

2. A tendency to increase the dose, though some patients may remain indefinitely on a stationary dose.

3. A psychological and physical dependence on the effects of the drug.

4. The appearance of a characteristic abstinence syndrome in a subject from whom the drug is withdrawn.

5. An effect detrimental to the individual and to society.

Drug Habituation (habit) is a condition resulting from the repeated consumption of a drug. Its characteristics include:

1. A desire (but not a compulsion) to continue taking the drug for the sense of improved well-being which it engenders.

2. Little or no tendency to increase the dose.

3. Some degree of psychological dependence on the effect of the drug, but absence of physical dependence and hence of an abstinence syndrome.

4. Detrimental effects, if any, primarily on the individual."

It has also defined sedative, hypnotic, tranquilliser and stimulant drugs but gives both pharmacological and popular conceptions of the term "narcotic"—to the man in the street "drug" and "narcotic" are often understood as drugs of addiction.

It will be seen that addiction is differentiated from habituation in that in the latter there is less tendency to increase the dose and absence of physical dependence. This distinction is not absolute—the report accepts the existence of the stabilised addict and indeed gives brief case histories of six such people who take their share in the work of the world without increase of the dosage on which they are dependent for freedom from pain. Where drugs are used in this way for relief from chronic pain some authorities maintain that it is wrong to regard the sufferer as an addict. Again the habitué may find that his intake of tranquilliser or barbiturate increases yet he may not suffer from physical dependence, or only to the extent to which the smoker exhibits such dependence when deprived of his cigarettes. "Habituation" is also used in another sense by Wikler (1961) as a synonym for relapse after cure, but such use of the word might lead to confusion.

Tolerance

The British National Formulary 1960 in referring briefly to habit-forming drugs gives the warning "In a susceptible person drug tolerance can readily develop and will reveal itself by a call for increased or more frequent dosage to obtain the required clinical effect". The nature of tolerance has been much disputed; it is not primarily a question of the better or quicker metabolism or excretion of the drug, though Kato (1961) has demonstrated that meprobamate and phenobarbitone produce even within a day an increase of activity in the liver's drug-metabolising enzymes which break down meprobamate. The fact that tolerance to morphine is developed to its depressant but not to its excitatory effects led to a hypothesis that addiction developed to mask the cumulative effect

of residual excitation that persisted when the depression had worn off, but the phenomena of the abstinence syndrome do not support this theory.

While the precise nature of tolerance remains undetermined it is believed to be a cellular phenomenon—cells acquire the ability to survive and function in the presence of concentrations of morphine (or alcohol) which would ordinarily inactivate them, rather as trypanosomes can be accustomed to flavines. This must apply also to barbiturates and other hypnotics although the degree of tolerance that can be developed to opiates greatly exceeds that to most other narcotics.

Interim Report

In November, 1959, the Interdepartmental Committee submitted an interim report on two problems specially referred to it by the Ministers. The first was the occasional misuse of carbromal and bromvaletone and mixtures containing these drugs. The Poisons Board had repeatedly reviewed their growing use but felt they were not more toxic than such drugs as aspirin. We recommended that any drug which so affected the central nervous system as to be liable to produce physical or psychological deterioration should be supplied only on prescription and this led to the modifications in The Poisons Rules, 1960. It is hoped that when new drugs with comparable actions are introduced, they will quickly be similarly scheduled. Such delays as followed the introduction of pethidine might well be disastrous.

The second difficulty arose over anaesthetists who became addicted to the gases and vapours they use. Examples of such abuse which might endanger the lives of their charges had recently come before Courts of Law, and while anaesthetic experts regard a preliminary sniff at their mixtures as an indispensable precaution we recommended that the addict should never be allowed to administer anaesthetics and that the anaesthetist's professional colleagues should intervene in any such case. Appropriate steps have been taken by the authorities to implement these recommendations.

Synthetic Analgesics

The Rolleston Committee met before the problem of synthetic analgesics had arisen, apart from derivatives of morphine such as diamorphine. Experience has since shown it to be unlikely that a potent analgesic will be free from addicting potentialities. Facilities for testing these on man are not available in Great Britain but exist at Lexington in the U.S.A. There would appear to be degrees of dangers of addiction even amongst very potent analgesics, for example, phenazocine has been introduced with the claim that it is less of a menace than morphine. The establishment of such a distinction is only possible after prolonged clinical trial, although the W.H.O. experts (1962) are studying both the experimental and clinical methods by which the addicting potentialities of a drug may be investigated and assessed.

In America there is strong opinion that the synthetic analgesics have now been so developed that the opiates can be dispensed with entirely—

we in Great Britain do not subscribe to this and still pay homage to "the incomparable morphine" even if its addicting tendency be greater and the treatment of any such addiction more difficult than those of most synthetic substitutes. We can point with reasonable confidence to our Table of Addicts and without being smug say "Ours is not the problem with which you, in America, contend".

TABLE I

EXTENT, TRENDS AND NATURE OF THE ADDICTION PROBLEM IN GREAT BRITAIN. ADDICTS KNOWN TO THE HOME OFFICE

Drug	1936	1950	1960
All drugs	616	226	454
Morphine	545 (88 per cent)	139 (61 per cent)	204 (45 per cent)
Pethidine	—	34 (15 per cent)	116 (26 per cent)
Methadone	—	5 (2 per cent)	51 (11 per cent)
Levorphanol	—	—	16 (4 per cent)
"Professional" addicts — doctors, dentists, veterinarians and pharmacists	147 (24 per cent)	48 (21 per cent)	68 (15 per cent)

An examination of the figures available to the Interdepartmental Committee indicates something of the changes of the last 25 years (Table I). Much doubt has been cast on the accuracy of these Home Office figures—our transatlantic friends view them with envy not unmixed with frank disbelief. When Sir Russell Brain (1961) discussed the report of his committee at a meeting of the Society for the Study of Addiction last year he was taken to task for his optimism by a pharmacist who claimed he could "record 40 or 50 cocaine, heroin and morphine addicts in the London area alone" and told of one, unknown to the Home Office, who was presenting prescriptions supplied by a doctor "who was making every effort to treat these people" for "something like 30 grains of cocaine or 40 to 50 grains of heroin". He claimed that such consumption was evidence that this patient had "been obtaining supplies illicitly to get used to these quantities". There are, of course, likely to be a few addicts whose records have not yet attracted the attention of the authorities but the opinion is that they are few—possibly recent arrivals in this country and it is feared that the treatment threatened or meted out to the addict in some countries may on occasion drive him to Britain. But the U.S.A. has 50,000 morphine addicts, 10,000 of whom are juveniles. We can be confident that there is no addiction on any comparable scale in Britain. The disparity may be accounted for by the British subject's law-abiding tendencies and respect for the law, the careful way in which the law has been interpreted and administered and of course the careful way in which these drugs are handled.

This pharmacist's experience of a large number of addicts in London emphasises another trend. Addicts are generally found in large centres of population; Isbell emphasises that the addicts of U.S.A. are substantially concentrated in certain areas of New York, Philadelphia, Chicago and Los Angeles. He also maintains that these unfortunates are mostly

psychopaths from the depressed strata of society. It would therefore seem specially important that the psychopath should never be introduced to potential drugs of addiction where this can be avoided.

Habit-Forming Drugs

Apart from a few individuals whose personalities may well be more responsible for their addictions than the drugs they take, we think of habituation rather than addiction to sedatives, hypnotics, tranquillisers and stimulants. There is also habituation to the milder analgesics—Fourneau's antalgiques—and especially where the coal-tar derivatives are combined with codeine. Codeine has recently been commented upon by the W.H.O. Expert Committee on Addiction-producing Drugs (1962). Consumption of codeine continues to increase and this is thought to be less due to its antitussive use, for many synthetic antitussives have been introduced lately, than to compounded analgesic preparations (Analgin, Antoin, Cephacan, Codis, Dellipsoids D-4, Dexocodene, Dolviron, Hypon, Nembudeine, Neurodyne, Pardale, S.A.C., Vagadil-Alk, Veganin).

TABLE II

Relative consumptions of pethidine and morphine in selected countries

	Pethidine used (in kg.) in 1960	Ratio (per cent) of pethidine to morphine used in 1960
U.S.A.	10,758	0·63
United Kingdom	2,745	0·16
Germany	1,497	0·13
Canada	638	15
France	500	0·05
Ireland	66	5
Mexico	56	19

Codeine has a relatively low addiction liability, we are assured, and "its use will be advantageous as long as it prevents the use of substances of higher addiction liability. Its use will be hazardous if it leads to a habit of drug administration and induces substitution of a more dangerous drug". Self-medication can so easily become a habit. Much energy has been directed by the manufacturers to the evolution of something better than codeine and the compound codeine tablet and the use of tranquillisers outside of hospitals may be a matter of some anxiety, especially in view of the side-actions associated with these drugs. A few years ago there was an attempt to popularise the use of rauwolfia preparations as a drug for free sale—fortunately and perhaps partly on the advice of the Pharmaceutical Society's Council and the brave action of the lamented "Chemists' Federation" this was withdrawn before it was established and long before it was appropriately scheduled as a poison. No one can deny the value of reserpine when properly used and controlled, nor that of the numerous phenothiazines which have so much affected behaviour and prognosis amongst the mentally sick. The Report quotes the ten-fold increase in chlorpromazine consumption in nine selected mental hospitals over 5

years—fortunately most practitioners treat these drugs with a healthy respect, valuable though they are in psychiatry.

The problem of habituation to barbiturates in Britain is a more serious one. Usage in England and Wales "has expanded both progressively and substantially so that in 1959 it was almost twice what it was in 1951". The barbiturate addict, well recognised in America, is still rare here but too many, especially amongst the elderly, drift into nightly dependence on their capsules or tablets and some acquire an almost new lease of life when weaned of their habit. Certain aspects of the problem should be noted:

1. An increasing number of barbiturate substitutes are being developed and advertised. Whether these represent any substantial therapeutic advances remains to be established. Lasagna (1957, 1962) has cast grave doubts on the merit of many. One promising substitute, thalidomide, has had to be withdrawn because of side-actions which had not become apparent even in prolonged and thorough clinical trials.

2. The regular use of sedatives at night may be a factor in the increasing use, as a *corrigens*, of such stimulants as amphetamines and phenmetrazine. The combination of sedative and stimulant has also been recommended and formulated—and has been found useful in spite of its "pharmacological incompatibility". Amphetamine addiction at one period reached alarming proportions in Japan but only 50 cases have been reported in this country. In an analysis of N.H.S. prescriptions numbering many millions, 1 in 40 was for these stimulants.

3. The increased consumption of barbiturates has led to a still increasing incidence of barbiturate poisoning to which much attention has been directed in the past decade. Many of these are cases of attempted suicide and probably not a few alleged accidental poisonings are suicidal rather than accidental. But there is no evidence that the possession of barbiturates is an encouragement to suicide; this country's suicide rate has not gone up even if barbiturates are now often preferred to coal gas or more dramatic, and more certain, methods.

The Future

What is to be done? After spending 2 years in reviewing a great deal of evidence, the Interdepartmental Committee may not appear to be very far-reaching in its recommendations. Perhaps the most important of these was that of the interim report—that any drug which is liable so to affect the nervous system as to produce physical or psychological deterioration should be supplied only on prescription. This puts the responsibility on the doctor. The doctor is advised to seek a second opinion if he feels that he must prescribe a prolonged course of dangerous drugs and to give only a limited supply of such to a patient temporarily under his care unless he has been in correspondence with the patient's own doctor. After weighing the pros and cons, proposals for the establishment of specialised institutions, compulsory committal of addicts to such, systems of registration of addicts, the use of special distinctive prescription forms for

dangerous drugs, further statutory powers to control new analgesic drugs or to cope with irregularities in prescribing are not regarded as necessary or desirable. The substantial increase in the use of drugs which are potentially habit-forming is regarded as something which requires careful watching but, at present, no further statutory control.

The Pharmacist

The pharmacist is the man who knows all about drugs and increasingly the doctor will lean upon him for guidance and be grateful for his advice. With the insistence on prescriptions for all drugs that are potentially habit-forming it may seem that the burden of responsibility is placed upon the doctor rather than the pharmacist. But the scrutiny of prescriptions for dangerous drugs has often led to the detection of errors, of wrong doses, of alterations made by the patient to increase supplies. No less important is the co-operation between doctor and pharmacist as two professional men, both part of the Health Service, both concerned with the welfare of those who seek their aid. Knowing the miseries that addiction can produce, both are concerned with avoiding the risk but this does not mean that they are unprepared to use dangerous drugs as necessary for the relief of pain. Provided the physician and pharmacist meet they will find ways and means of helping each other.

TABLE III

Barbiturates prescribed and incidence of poisoning in England

Year	Tons prescribed	Known cases, approx.
1938	20	40
1953	40 under N.H.S.	2,500
1959	80 under H.H.S.	6,000 (10 per cent mortality)

The responsibility is not limited to the retail pharmacist. The hospital pharmacist may have difficulties over the authority held by sisters and acting-sisters in charge of wards to hold stocks which the pharmacist has to check from time to time. True, the sister only supplies these drugs to patients "in accordance with the instructions of the doctor in charge" but drugs are not always checked as regularly as might be desirable. On the other hand some sisters insist on a daily personal check of their Dangerous Drugs cupboards.

The manufacturing pharmacist who may be concerned with the introduction of a new drug of potential addiction clearly has a great responsibility. If its dangers are not recognised and its distribution safeguarded from the first, great harm may be done. Much attention has recently been focussed on adequate clinical trials for new products. If the product be possibly addicting suitable tests are the more necessary and such have been devised and used at Lexington. We may have to refer our questions to such a centre.

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SUMMARY OF

SECOND INTRODUCTORY ADDRESS

BY J. M. JOHNSTON,
C.B.E., M.B., CH.B., M.D., F.R.C.S.ED., F.R.C.P.ED.

Advisory Committee an Medical Research, Scottish Home and Health Department Edinburgh, 1

THERE is no agreed scientific definition of addiction. There is a popular definition and it works: The patient says "I can't do without it."

There is an absence of precise scientific knowledge about addiction. From the pharmacological and clinical aspects, drug tolerance, habituation and addiction are part of a spectrum, and it depends where the dividing line is drawn as to whether one is dealing with an habituate, an addict, or merely one who had a tolerance. It is extremely difficult to say that one individual is habituated and another is addicted. More knowledge of enzyme reactions might provide a clue to the situation.

With addiction there is a tendency to increase the dose: with habituation, little tendency. There is also a psychological and physical dependence in addiction but psychological dependence only in habituation. The occurrence of an abstinence syndrome depends on the patient, on the amount of the drug, and on the duration of treatment.

In the range of habituation and addiction, the patient's symptoms remain unabated and his demands increase. Experience suggests that that state of affairs arises from an inability to cope with life—a form of escape from reality. There follows a depression of moral standards, and then a swift depression of morale.

Drugs which may lead to addiction are those which relieve anxiety, or tension, or fear, or all three. Morphine, heroin, pethidine and, perhaps, certain barbiturates, in proper hands, were most beneficient drugs but lead to addiction if misused by the patient or by the prescriber. New sedative, hypnotic, or tranquillising drugs might prove sooner or later to be addictive; it is not always possible to uncover these properties until some years have elapsed.

In most cases addiction begins with a clinical need. In the official returns for addicts, what may be termed "therapeutic addicts" were by far the biggest number in Great Britain—approximately 75 per cent of all cases.

The Dangerous Drugs Act has been a success, because we are a law-abiding population, with a law-respecting medical and pharmaceutical profession, an efficient law enforcement body, and a wise, and prudent team of men in the Home Office. In Great Britain, drug addiction is not a crime—the crime consists in the illegal obtaining of the drug.

The pharmacist is in a special position; he might notice certain types and categories of drugs being prescribed for particular patients where either the amount begins to rise or repetition becomes more frequent. Although certain patients might continue in a mild type of addiction for a long time, there are other factors to be considered such as the danger to others. Any person driving a car when under the influence of an addictive drug is a potential menace to himself and to others, his judgment, his work, and his reactions are all affected.

Although cures for addiction are frequently claimed, so far as dangerous drugs are concerned, relapses frequently occur. An addict can only be considered cured when for 5 years he has been able not only to do without drugs, but also has no desire for them. The patient should have the will to co-operate and a moral regeneration is required.

There appears to be no moral, scientific or professional reason why a given drug should be removed from therapeutics because in some countries it has been misused.

SUMMARY OF

THIRD INTRODUCTORY ADDRESS

BY B. J. THOMAS, M.P.S.

Allen and Hanburys Ltd., Bethnal Green, E.2

APART from alcohol, morphine and cocaine are the oldest established drugs of addiction, other drugs are the barbiturates, amphetamines, dihydromorphinone, levorphanol, pethidine, methadone and phenadoxone, while more recently, though to a lesser extent, addiction has occurred after taking methylpentynol, carbromal, bromvaletone and paraldehyde.

The craving for morphine may induce criminal behaviour in an attempt to obtain a supply. Heroin has a shorter duration of action than morphine and therefore prescriptions are presented more frequently than those for morphine. When introduced, pethidine was free from control and was hailed as the morphine substitute which did not produce addiction, and many of the present addicts acquired their habit as a result of taking the drug during this period. In the case of methadone,

the interval between its introduction and its inclusion in the Dangerous Drugs Schedule was fortunately much shorter.

Addiction to amphetamine and related drugs has been curbed as a result of legislation, but inhalers which are available without control are sometimes purchased in excessive and frequent quantity by addicts.

When regular requests are made for 2 or 3 bottles of vasoconstrictors at a time or when repeated quantities of chlorodyne are sought, the customer should be persuaded to seek medical advice, while joint action by all the pharmacists in the district in limiting supplies might further this aim.

Ether should never be supplied unless the pharmacist is satisfied that a *bona fide* reason exists for its use. Paraldehyde can become a drug of addiction and bromide intoxication still exists, but the total extent of drug addiction in Great Britain is not large. The pharamcist exercises considerable legal authority under the Pharmacy and Poisons Act, and the Dangerous Drugs Act, and together with the other persons entrusted with the control, manufacture, supply and prescribing of drugs of addiction, he shares a public and moral responsibility.

Discussion

On the invitation of the Chairman, Dr. Cedric Wilson opened the discussion by describing an experiment in which groups of patients in the Merseyside area were given either coloured or white tablets containing dexamphetamine and a barbiturate, or a placebo. Analysis of the patients answers to a questionnaire showed that a significant number were neurotic introverts. Sixty-one per cent stated that the "dummy" tablets were of benefit. The others were pharmacologically dependent on the drug and, on Professor Macdonald's definition, were habituated though he (Dr. Wilson) did not agree with this.

Other points made were:

Attention was drawn to the means adopted to extract drugs from ampoules in hospital practice; minute holes or cracks were induced, the drug extracted and the ampoule either refilled with water or claimed not to be full. It was observed that drugs available in hospital were solely for the treatment of patients in that hospital. Drugs which might lead to addiction were being obtained through laxity of Schedule 4B. Patients receiving amphetamines could be classified in four groups: (*a*) those who could give up the drug when treatment ceased, (*b*) those who had a dependence upon a drug not because it was needed but because it was thought to be needed, (*c*) those who found it necessary to increase the doses for therapeutic reasons, (*d*) those who had a compulsive need for a drug in increasing quantities and who were prepared to go to extreme lengths to get it. The latter group was considered to be very small. Official guidance was sought on the problem of recovery and handling of dangerous drugs from deceased patients and from those who no longer needed them. One procedure which had been found acceptable to some local authorities was the destruction of the drug by the pharmacist in the

presence of the local DDA officer who then issued a certificate stating what had been done. Guidance was also needed on the correct interpretation of the phrase "reasonable steps to ensure a prescription is genuine" and the term "possession" relating to the key of the DDA store. The possibility that barbiturate habituation was the result of a fashion in drug prescribing, coupled with a delay in stopping medication, was suggested. The continued use of cocaine was deprecated.

CONFERENCE LECTURE

PHARMACOGENETICS—A STUDY OF INHERITED VARIABILITY IN THE RESPONSE TO DRUGS

BY C. A. CLARKE, M.A., M.D., F.R.C.P.

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Multifactorial Inheritance

YOU know a lot about drugs and I know a little about genetics and I thought it might be interesting to marry up our two interests for a short while today. However, before we discuss this so-called pharmacogenetics I must remind you of the two principal ways in which characters are inherited. On the one hand they may be controlled by many genes (multifactorial inheritance) and then, according to whether you have a few, a medium number or the complete set so will you manifest more or less of the trait in question. The usual example given is that of human height where you can be anything from very short to very tall but with a likelihood of being somewhere around 5 ft. 8 in., assuming the nutritional side of the matter to be adequate throughout.

Single Gene Inheritance

On the other hand, some characters are all-or-none, that is, you either have them or you do not and these are controlled by single genes which you either do or do not possess. A good example of single gene inheritance in normal people is afforded by the blood groups. Thus you are either group A *or* group O *or* group B *or* group AB and there is no grading.

Polymorphism

The next concept that I want to introduce to you is that of genetic polymorphism. The best way to explain it is by taking an example. Let us again consider the blood groups. Now the various components of the system I have mentioned exist in certain frequencies. Thus here in Liverpool about 50 per cent of people are group O, 40 per cent A and 10 per cent B or AB. Since geneticists think that genes are never entirely neutral as regards survival value there must be balancing forces which keep the members of any polymorphic system, such as the blood groups, in equilibrium, otherwise one of them would increase at the expense of the others and in due course the most advantageous would be the only one left. On reflection it is clear that susceptibility to diseases of one sort or another might well be an important factor in stabilising this equilibrium, and in fact it has been found that people who are group A are slightly more prone to gastric cancer than the other groups and that duodenal ulcer patients are particularly likely to be group O. It must be remembered, however, that factors responsible for maintaining genetic equilibrium must operate during the reproductive period for the effects of selection to be passed on. If you die of duodenal ulcer aged 60 it will

not have affected the frequency of group O in the next generation because you will have had your children by then. If you die of the disease aged 20 then there *will* be a drain on group O. It only needs a moment of reflection to see that the effects of duodenal ulcer and gastric cancer on maintaining the equilibrium of groups O and A are very small.

There are fortunately much more easily understood examples of factors that stabilise polymorphisms and if we turn to some of our humbler relatives in the animal kingdom we find that they are most informative. The British peppered moth exists in two forms, "typical" and *carbonaria*. The two patterns are inherited and a single gene converts "typical" to *carbonaria* and there are no intermediates. A hundred years ago the whole population in England was "typical"; then in Manchester about 1850, there arose the *carbonaria* and it has spread so rapidly that at the present time in certain areas 95 per cent are *carbonaria*; however, nowhere has the "typical" form been completely eliminated. *Carbonaria* and "typical" represent a polymorphism just like the blood groups and one of the reasons why the change has taken place is the better camouflage of the black form in areas affected by industrial pollution. In the moth, therefore, a change in environment has altered a genetic situation favouring those insects of a particular genetic constitution which previously had enjoyed no advantage. We shall see later that there is a parallel in Man.

Salicylic Acid and Isoniazid

Now when we come to consider the response of the body to drugs the two types of inheritance that I have mentioned hold good. For example, if we consider the metabolism of salicylic acid we find that on administration of a standardised dose to 100 healthy individuals we obtain a normal distribution for the serum salicylic acid 3 hr. after the

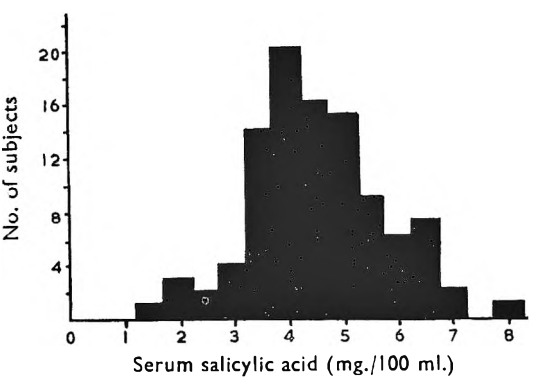

FIG. 1. Serum concentrations of salicylic acid 3 hr. after ingestion of 50 mg. of sodium salicylate per kg. of metabolically active mass (= $wt.^{0.71}$).

drug ingestion (Evans and Clarke, 1961), and this is consistent, as far as genetics is concerned, with the multifactorial inheritance that I mentioned earlier in which most of the individuals fall into the middle grades (see Fig. 1). All a curve such as this suggests is that the individual

variation is continuous and it is not a promising situation to investigate either from a genetic or an environmental point of view.

The normal curve probably holds for the majority of compounds but occasionally we meet with the single-gene, all-or-none situation where there is a clear-cut difference in the way a drug is metabolised. The best example is isoniazid. Here we find that, in this country, the drug is inactivated by acetylation rapidly by 50 per cent of the population and slowly by the other 50 per cent and that there are very few intermediates. Furthermore, family studies have shown that the situation is controlled by a single pair of genes, "rapid" being dominant to "slow" (Evans, Manley and McKusick, 1960) (see Fig. 2).

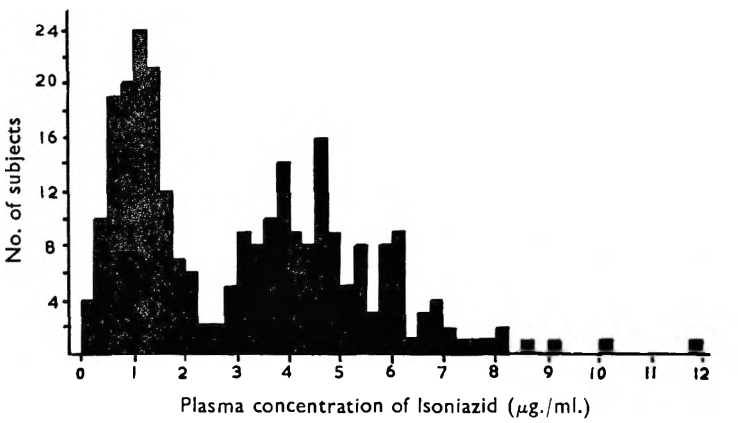

FIG. 2. Plasma concentration of isoniazid 6 hr. after oral administration of 9·7 mg./kg. body weight. There were 53 families, 267 family members.

Several things follow from this knowledge. First, you can use isoniazid as a genetic marker, just like the blood groups, but this is not pharmacologically very interesting. Second, and much more stimulating, is to consider whether the disease for which the drug is used—in this case tuberculosis—is modified by what we may call the "inactivator status" of the individual. *A priori* we might think that the "slows" would benefit more from the compound than the "rapids" and there is a suspicion that this may be so. However, the effect is not a big one and is not likely to be of much importance from the practical point of view of the management of the disease. Nevertheless, when we come to look at the toxic effects there is much more to it—the "slows" are heavily loaded compared with the "rapids" in their liability to develop polyneuritis (Devadatta, Gangadharam, Andrews, Fox, Ramakrishmam, Selkon, and Velu, 1960) (see Table I). It would also be interesting to look at the response to the drug of lupus vulgaris, which is often treated by isoniazid alone, and it would be easy to measure the size of the lesion and assess its rate of disappearance in relation to the "inactivator status" of the patient.

Thirdly, we can look at the matter from the much broader biological point of view to try and find out the balancing advantages and disadvantages of being "rapid" or "slow." We have actually no idea what

factors are involved but they must be different in different parts of the world, because in Japan and in the Eskimos (Harris, Knight and Selin, 1958) 90 per cent of the population are "rapid" and 10 per cent "slows" whereas in Africa there are more "slows" than "rapids." Therefore, it seems likely that it is more advantageous in northern latitudes to be "rapid"—though not overwhelmingly so because 10 per cent of people there still remain "slow." It is clear that the metabolism of isoniazid *per se* is unlikely to have been a stabilising factor in earlier times since the drug has only been recently discovered, though it might become important in the future. It is, however, possible that in the past we ate naturally occurring, chemically related compounds possessing anti-tuberculosis activity and that these may have been metabolised in the same way as isoniazid. If this were so it would cause the "slows" to be preserved in populations which were particularly exposed to the disease.

TABLE I

THE DEVELOPMENT OF POLYNEURITIS ON ISONIAZID THERAPY

Inactivator phenotype	Without polyneuritis	Polyneuritis	Totals
Rapid	58	2	60
Slow	66	17	83
Totals ..	124	19	143

$\chi^2 = 8{\cdot}87.$ $p < 0{\cdot}01$

Dr. D. A. Price Evans (1962 and in preparation), of the Department of Medicine of the University of Liverpool, has now advanced the understanding of the difference between the rapid and slow inactivators of isoniazid by *in vitro* experiments making use of the enzymes of the liver which carry out the process of acetylation. This they do by enabling acetyl coenzyme A to give up its acetyl radical to an acceptor molecule, for example, a drug. Biopsy specimens of liver were ground up, suitable additions made and the homogenate then mixed with isoniazid. After a given period the amount of free drug was estimated and from this the amount acetylated was calculated. The result showed clearly that sometimes the isoniazid was acetylated slowly and sometimes quickly, and this tallied with the "inactivator status" of the patient from whom the liver specimens were obtained.

The great advantage of being able to carry out such an investigation *in vitro* is that it becomes possible to find out exactly which enzymes are responsible for particular metabolic steps.

In a second experiment, the amount of acetylation of sulphadimidine by volunteer medical students given a standard dose of the drug was estimated in urine collected for 8 hr. after the drug was taken. Dr. Price Evans found that there was a clear bimodality in the acetylation of the drug, some of the students carrying it out quickly and others slowly, and that the rapid acetylators of sulphadimidine were also the rapid inactivators of isoniazid and the same was true of the "slows", so it looks as if the same gene is controlling the metabolic step in each case. Now what follows from this?

Dr. Price Evans feels that the enzyme basis for these acetylation polymorphisms lies either in the rate of transfer of acetyl groups from acetyl coenzyme A to the substrate (drug) molecule; or alternatively in the rate of production of acetyl coenzyme A itself. Only further experiment will be able to decide which is in fact the case, but the matter generally seems of great interest.

Primaquine

We next turn to another drug, namely primaquine, which has been found to uncover a most interesting genetical situation. Let us begin at the beginning. Haemolytic anaemia was recognised as an occasional complication of the now out-of-date pamaquine when it was introduced in 1925 as an effective agent against the sexual forms of the malarial parasite, *Plasmodium vivax*. At first the anaemia was thought to be

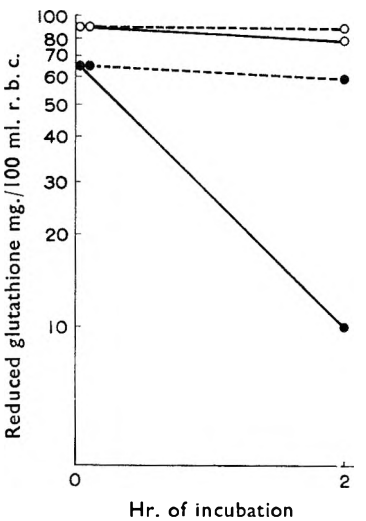

FIG. 3. The effect on reduced glutathione of incubating sensitive and non-sensitive red cells in the presence of glucose (Beutler, 1960, by kind permission of McGraw Hill Book Co.).

○ - - - - ○ Non-sensitive red cells incubated without primaquine.
○ ——— ○ Non-sensitive red cells incubated with primaquine.
● - - - - ● Sensitive red cells incubated without primaquine.
● ——— ● Sensitive red cells incubated with primaquine.

due to a hypersensitive or immune mechanism but no antibody was ever discovered and the problem remained unsolved until many more cases occurred during World War II when the very similar drug primaquine came extensively into use. We must next discuss the clinical features of this anaemia which are of some interest. A sensitive subject when given 30 mg. of primaquine daily does not develop the blood changes for 2 or 3 days. Thereafter his urine gradually turns dark, muscular pains occur and anaemia and possibly jaundice appear. Discontinuing the drug results in a return to normal over a few weeks. However, if

the symptoms are not severe and primaquine ingestion is continued he will, surprisingly, also improve. This is a most important observation and the reason for it was discovered by labelling red cells of different ages with radioactive iron (Dern, Weinstein, Leroy, Talmage and Alving, 1954). This has shown that red cells in a sensitive subject can be lysed by primaquine when 63 to 76 days old but not when 8 to 21 days old Therefore, it seems that it is the ageing erythrocyte which is destroyed by primaquine, and spontaneous clinical recovery, while continuing to take the drug, is due to the regeneration of a red cell population with a low mean age.

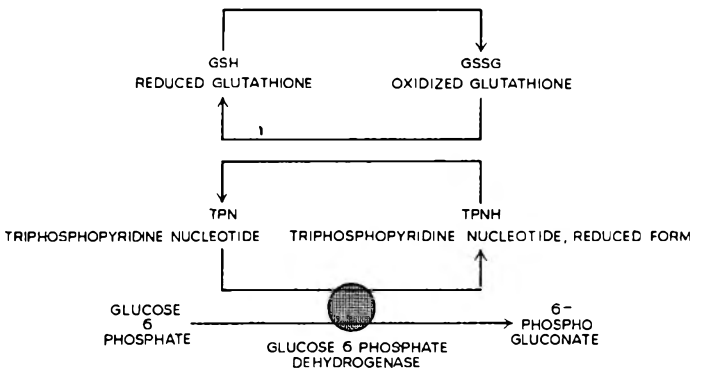

FIG. 4. The hydrogen atom removed from G6P by G6PD is taken up by TPN and TPNH is formed. This in turn reduces GSSG to GSH. If G6PD is deficient, the cycle is interrupted and no GSH is formed.

These are the facts regarding primaquine and we must turn to biochemistry for the explanation. The normal red cell possesses enzyme systems which are concerned with the metabolism of glucose. One of these is glucose-6-phosphate dehydrogenase and the important finding is that this is greatly diminished in primaquine-sensitive individuals. The first observation which led to this discovery concerns reduced glutathione (Childs and Zinkham, 1958). Thus when both primaquine-sensitive and non-sensitive red cells are incubated with the drug *in vitro*, with glucose added to the buffer, the content of reduced glutathione falls in the cells from sensitive individuals but not in those from normals. The continued fall in sensitive red cells is due to a fault in glucose metabolism, the result of a defect in glucose-6-phosphate oxidation brought about by a deficiency in the appropriate dehydrogenase (G6PD) and more marked, as stated above, in the older cells. The two diagrams (Figs. 3 and 4) give the details of the normal and faulty mechanisms.

Glucose-6-phosphate Dehydrogenase Deficiency

The next point to appreciate is that the lack of G6PD and hence the presence of primaquine-sensitive individuals is an inherited characteristic, and males are readily assignable either to the sensitive or non-sensitive group on both glutathione and direct G6PD studies. Women do not give

so clear-cut a division and intermediate values are observed with both methods of assessment. These sex differences together with pedigree analysis, led to the conclusion that the gene controlling the presence or absence of G6PD is sex-linked, that is, on the X chromosome, and that the trait is semi-dominant to its normal partner (Childs and Zinkham, 1959). In women with two Xs only one carries the abnormal gene and the other lessens its effect. This is not the case in men who will have either a normal or an abnormal X with a clear-cut result in either event.

The important point to appreciate is that primaquine has divided individuals into two groups, non-sensitive and sensitive and the latter can comprise as many as 10–15 per cent of some populations, for example, in certain Mediterranean countries, Persia, parts of India and Indonesia, though in England sensitivity is excessively rare. When we consider the world distribution of G6PD deficiency it is seen that a high frequency coincides with a high incidence of malaria and there is now additional evidence that G6PD deficient cells are less favourable to the proliferation of the malarial parasite than are normal ones (Allison and Clyde, 1961). This is an overriding advantage compared with the possibility of developing haemolytic anaemia from primaquine and incidentally from other substances as well. For example, the bean *Vicia fava* produces a similar syndrome in G6PD deficient individuals and so also may naphthalene mothballs—children sometimes eat them or they may be taken by adults in pregnancy as a perversion of appetite.

Hydrogen Peroxide and Acatalasia

We next come to a very ordinary drug, so mundane indeed that I doubt whether an audience such as this would deign to call it a drug at all—hydrogen peroxide. Now the polymorphism which this has uncovered is of particular interest to me because it shows that the art of clinical observation is neither dead nor useless. In 1946 a Japanese oto-rhino-laryngologist noticed that the operative field in a patient from whom he was removing a maxillary antral tumour turned black and did not froth when he dropped hydrogen peroxide on to it; normally, of course, the raw surface of a wound does froth and the blood does not alter in colour. It was found that the blood of this particular patient lacked an enzyme called catalase which normally degrades hydrogen peroxide and prevents the oxidation of haemoglobin by peroxide. Relatives of the patient were next investigated and it was found that some of them also lacked catalase. These people, usually children, lose their teeth and develop necrosis of the jaw. This is because haemolytic streptococci and certain pneumococci themselves produce hydrogen peroxide, and in a patient lacking catalase the haemoglobin of the blood reaching a lesion is oxidised so that necrosis occurs since the infected area is deprived of oxygen. In this situation the bacteria multiply, the hydrogen peroxide production increases and a vicious circle is established. Several points of interest about the syndrome are now known. Only about half of those who lack the enzyme actually show symptoms, and once all the teeth have been removed the lesions heal and many patients

remain permanently free of trouble. A similar condition has also been found in certain breeds of dog and guinea-pig (Wyngaarden and Howell, 1960; Allison, Rees and Burn, 1957).

It seemed not unlikely that when the condition was better known cases would be found in countries other than Japan, and recently it has been described in Switzerland. Acatalasia has been found, by pedigree studies, to have a recessive method of inheritance.

Suxamethonium Sensitivity

We now turn to anaesthetics. It has been known for some years that patients given suxamethonium to ensure muscle relaxation during anaesthesia have occasionally been unduly sensitive to the drug. Prolonged apnoea occurs and although the breathing can almost always be restored yet the situation is temporarily alarming. The reason for this is as follows. Normally suxamethonium is broken down by the serum cholinesterase but in sensitive individuals the value for the activity of this enzyme is low, and the same is true for some of the relatives of those known to be affected. On further investigation of those who tolerated the drug badly it was found that the enzyme present was different from that in normal individuals—it was not just a question of producing normal cholinesterase in smaller amounts than usual. Now the activity of the usual type of serum cholinesterase can be assessed quantitatively. The local anaesthetic drug cinchocaine (dibucaine) is a convenient inhibitor of the hydrolysis of benzoylcholine by the enzyme. The percentage inhibition so produced is called the "dibucaine number", and in normal people it is about 80 whereas in those affected it is around 16. A third group where the value is about 62 represents carriers of the condition (Lehmann and Ryan, 1956; Davies, Marton and Kalow, 1956). The condition has a recessive method of inheritance.

Presumably both in suxamethonium sensitivity and in acatalasia there is some advantage in having the gene in single dose, otherwise the polymorphism would never have developed.

Phenylthiocarbamide (PTC)

You might think from what I have said that biochemical explanations of genetic polymorphisms are readily forthcoming, but in fact the examples I have given you are highly selected and in many cases we are in difficulties. Take for instance what I will call the PTC story. Phenylthiocarbamide (PTC) was discovered in 1932 and it was soon found that 75 per cent of people in this country could taste it in solution whereas the other 25 per cent could not—in other words, here again we have an inherited polymorphism and "tasting" is a mendelian dominant to "non-tasting" (see Fig. 5). What are the advantages and disadvantages of belonging to one of the two classes? We still do not know the answer to this but because PTC is chemically allied to some of the anti-thyroid drugs, thioracil in particular, it was thought worth while investigating the "taster" status of patients with thyroid disease and sure enough it was found that certain types of goitre had a considerably higher incidence of non-tasters than

did controls (Harris and others, 1941; Kitchin and others, 1959). Since goitrogens similar to PTC are present in certain foodstuffs, particularly vegetables such as cabbage, it was thought that dietetic and gustatory habits might have something to do with the maintenance of the polymorphism and it was clear that the next step was to investigate various substances with the chemical linkage $S = C\langle^{N=}$ to see if there were any clear-cut differences in their metabolism as between tasters and non-tasters. The short answer to this is that there are not. My colleague Dr. D. A. Price Evans has investigated the matter using the output of methyl thiouracil in the urine and also the uptake of thiopentone from the blood and in both he found no significant difference between tasters and non-tasters (Evans, Kitchin and Riding, 1962). It may be therefore, that the answer lies more superficially and that there are differences in the salivary enzymes between the two classes and this in fact is being investigated at the present time.

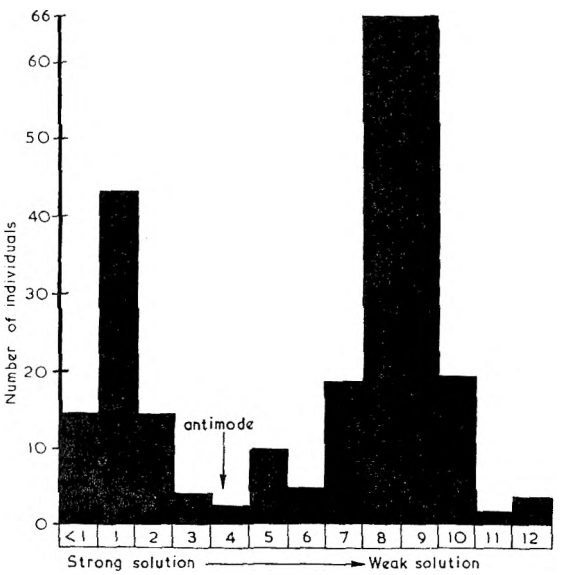

FIG. 5. Distribution of PTC taste response in 265 normal individuals (Kitchin and others, 1959).

Miscellaneous Information

I want to conclude by bringing to your notice very briefly some miscellaneous information.

Porphyria. Many forms of this disease are inherited and there are large numbers of afflicted individuals in Port Elizabeth in South Africa. Many of them used not to have much disability but since the introduction of barbiturates the matter has become much more serious since pentobarbitone in particular is extremely dangerous and because of this it is now the practice in Port Elizabeth always to test the urine for porphyrins before

giving this anaesthetic (Dean and Barnes, 1955). The porphyria story is a striking example of how a commonly used group of drugs can unfavourably influence an inherited disease.

Atropinesterase in rabbits. In some rabbits there is present in the serum an enzyme called atropinesterase. The ability to form this is genetically controlled and some strains possess it whereas others do not (Sawin and Glick, 1943). It would be interesting to know whether the enzyme is of any use to the rabbit by enabling it to inactivate solanaceous alkaloids, such as those contained in deadly nightshade and other plants. Is there anything comparable in Man? Confusional states occasionally occur after the administration of atropine eye-drops and it may be that different people metabolise the drug in different ways but whether there is an actual polymorphic system we do not know. Another interesting fact is that the mongoloid idiots are particularly sensitive to the effect of atropine.

Variations in pupillary responses. Blue-eyed Europeans are a little more sensitive than brown-eyed to the action of hydroxyamphetamine and other sympathomimetic drugs (Wells, 1958). The deep brown iris of the Negro is said to dilate only very little under the influence of these drugs. Here again therefore is a situation which would be worth investigating, particularly as nowadays many of the hypotensive drugs have ocular manifestations as one of their side effects, and it may be that patients with a particular eye colour are unduly sensitive.

I think you can be left in no doubt that pharmacogenetics is capable of shedding considerable light on normal biochemical processes. Moreover, when it comes to the question of drug trials, more attention should be paid to the possibility that patients may deal with a drug in two or more distinctly different ways rather than manifest minor individual variations.

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SCIENCE PAPERS

AN INVESTIGATION OF THE METABOLISM OF NEOSTIGMINE IN PATIENTS WITH MYASTHENIA GRAVIS

By Carol A. Scott, P. T. Nowell and A. Wilson

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Evidence of two metabolic products of neostigmine has been obtained by paper chromatography of urinary extracts from patients receiving the drug. One product has been identified as *m*-hydroxyphenyltrimethylammonium bromide and the relevance of this is discussed in relation to the therapeutic effect of oral neostigmine in the treatment of myasthenia gravis. A method is described for extracting neostigmine and related compounds from urine by precipitation with bromine water.

While studying the urinary excretion of neostigmine in patients with myasthenia gravis it was found that up to 67 per cent of the drug was excreted unchanged when given by intramuscular injection but only about 5 per cent when administered orally (Nowell, Scott and Wilson, 1962). This evidence suggested that after oral administration the drug is metabolised; one probable metabolite being *m*-hydroxyphenyltrimethylammonium bromide, attempts were made to identify this and any related substances in the urine of myasthenic patients.

Experimental and Results

Procedure

Urine (100 ml.) was evaporated to dryness and the residue extracted with absolute ethanol (2 × 10 ml.). The extract was centrifuged, evaporated to dryness and while still warm was dissolved in distilled water (2 ml.). To this solution bromine water (8 ml.) was added and after thorough mixing, the orange-yellow precipitate* was centrifuged off, and washed with 2 ml. water. The precipitate was then warmed with 50 per cent (v/v) methanol (5 ml.), centrifuged and extracted once more with 50 per cent methanol (1 ml.). The combined supernatant methanol extracts were passed through a column of Amberlite CG 50 resin (12·0 × 0·7 cm.), buffered at pH 6·86 with 0·2M phosphate buffer, containing 15·76 g. anhydrous Na_2HPO_4 and 15·11 g. $NaH_2PO_4.2H_2O$ per litre, and suspended in 50 per cent methanol. The column was washed with 50 per cent methanol (10 ml.) and then with water (10 ml.). It was eluted with 0·2N HCl (50 ml.) and the eluate evaporated to dryness. The residue was extracted with absolute ethanol (5 ml.), the extract was centrifuged and again evaporated to dryness. The residue was re-extracted with abolute ethanol (1 ml.) and 0·5 ml. of this solution was chromatographed on Whatman 541 paper using butanol : ethanol : water : acetic acid (8 : 2 : 3 : 0·25) as running solvent (Nowell, Scott and Wilson, 1962).

* The reaction involves the perhalides of quaternary ammonium nitrogen compounds and of brominated phenols.

Detection of Neostigmine and Related Compounds in the Urine of Patients with Myasthenia Gravis treated with Neostigmine

Urine was collected from six patients with myasthenia gravis before and after administration of a dose of neostigmine. Three of the patients were given 30 mg. neostigmine bromide by mouth and three were injected intramuscularly with doses of 2·5 mg. neostigmine methylsulphate. Each specimen of urine was extracted and chromatographed by the procedure described above.

With patients given intramuscular neostigmine, urine samples collected 2 and 4 hr. after administration yielded a spot with the same R_F value as neostigmine (0·50–0·53). This was absent in an 8 hr. sample. Each sample of urine also yielded three spots between R_F 0·2 and 0·35 but as these were present before treatment and also in normal urine, they were considered not to be derived from neostigmine.

By contrast, after oral administration of the drug, no neostigmine was detected in any samples of urine but a spot appeared at R_F 0·45-0·46 in specimens collected at 4, 8 and 12 hr. after treatment. Another spot was also detected at R_F 0·37–0·38 in the 8 and 12 hr. samples. The fact that these two spots gave a blue colour with iodoplatinate and had an alkaline reaction with bromocresol purple (0·1 per cent) in acetone/ethanol, 9:1, (Gordon and Hewel, 1955) suggested that they were quaternary nitrogen bases.

The substance at R_F 0·45–0·46 produced a characteristic blue colour with iodoplatinate which was different from the colour of all the other spots, but was identical with that obtained from an authentic specimen of *m*-hydroxyphenyltrimethylammonium bromide which when chromatographed alone had an R_F value of 0·48–0·51; when mixed with neostigmine and chromatographed, neostigmine was detected in its usual position (R_F 0·50–0·53) while the spot for the *m*-hydroxytrimethylammonium bromide appeared about 2 cm. lower, at R_F 0·45–0·46. This shift in position was also obtained when it was chromatographed in the presence of NaCl, the shift being independent of the amount of salt added. When *m*-hydroxyphenyltrimethylammonium bromide was added to normal urine a spot with the characteristic blue colour was obtained at R_F 0·45–0·46; suggesting that the spot seen at these R_F values after oral neostigmine was probably due to *m*-hydroxyphenyltrimethylammonium bromide. This conclusion was supported by the results of paper electrophoresis; similar extracts of urine from myasthenic patients treated with oral neostigmine produced a spot with the same mobility as that obtained from normal urine to which *m*-hydroxyphenyltrimethylammonium bromide had been added.

Discussion

The results of these experiments have shown that neostigmine is metabolised in the body, and that after oral administration two derivatives are excreted in the urine. One of these is probably *m*-hydroxyphenyltrimethylammonium bromide, the other (R_F = 0·37–0·38) has not

been identified, but is apparently a quaternary nitrogen compound also. The extent of this metabolic change cannot at present be measured quantitatively. The method of estimating neostigmine in urine, by formation of a complex with bromophenol blue, is not applicable to *m*-hydroxyphenyltrimethylammonium bromide because this forms a complex only at a concentration of about 1 mg./ml. which is much higher than would occur at any time in the blood or urine of patients receiving the normal therapeutic doses of neostigmine.

Work is at present in progress to determine the sites in the body where neostigmine is metabolised to *m*-hydroxyphenyltrimethylammonium.

These findings may provide some explanation for the apparent anomaly that after intramuscular injection, neostigmine is excreted unchanged in the urine but no unchanged drug can be detected after oral administration, although in each case satisfactory relief of signs and symptoms occurs. There is adequate evidence for the anticurare action of *m*-hydroxyphenyltrimethylammonium bromide (Cowan, 1938; Randall, 1950; Randall and Lehmann, 1950; Riker and Wescoe, 1950a; Riker and Wescoe, 1950b; Artusio, Riker and Wescoe, 1950; MacFarlane, Pelikan and Unna, 1950). The last mentioned authors have also shown that this substance effectively relieves the symptoms of myasthenia gravis; they reported that after an intravenous injection of 5 mg. *m*-hydroxyphenyltrimethylammonium bromide the therapeutic effect was equivalent to that produced by 0·4 mg. neostigmine, but was much more rapid in onset.

Acknowledgements. It is a pleasure to acknowledge the co-operation of the consultant physicians and surgeons of the United Liverpool Hospitals and Regional Hospital Board who provided us with the opportunity to study patients with myasthenia gravis. We are also grateful to Dr. J. A. Simpson and Dr. I. T. Draper, University Department of Neurology, Edinburgh, for providing specimens of urine from patients under their care. We wish to thank the Distillers Company, London, for their generous financial support wich provided one of us (C.A.S.) with a maintenance grant. The work was also supported by a grant from the Myasthenia Gravis Foundation Inc., New York. We are indebted to Messrs. Roche Products for specimens of *m*-hydroxyphenyltrimethylammonium bromide.

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The paper was presented by DR. NOWELL.

Short Communication

SOME EFFECTS OF A HEMICHOLINIUM COMPOUND (HC-3) ON NEUROMUSCULAR TRANSMISSION IN THE CAT

By E. R. Evans and H. Wilson

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Received May 23, 1962

The hemicholinium compound 4,4′-biphenylylenebis[carbonylmethyl-(2-hydroxyethyl)dimethylammonium bromide] (HC-3) (Long and Schueler, 1954) has been shown to produce a neuromuscular block in rabbits which is of gradual onset, long duration (Reitzel and Long, 1959) and dependent on the frequency of nerve stimulation (Wilson and Long, 1959).

An attempt has been made in the present study to determine the effect of HC-3 on neuromuscular transmission in the cat by using the tibialis anterior muscle-sciatic nerve preparation. Some effects on this preparation have already been described by Bowman and Rand (1961).

Experimental

Method

Cats (2 to 5 kg.) were anaesthetised with intraperitoneal pentobarbitone (35 mg./kg.) and the hind limbs supported in a Brown-Schuster myograph. The tendon of each tibialis anterior muscle was attached to a flat steel spring and the movements recorded on smoked kymograph paper. Twice maximal rectangular pulses of 0·05 msec. duration were applied to the cut sciatic nerve by silver electrodes. In experiments in which innervated muscles were stimulated directly, the stimulus duration was 0·10 msec. The blood pressure was recorded from the carotid artery by a mercury manometer and the vagus nerves were cut in the neck. HC-3 was administered intravenously.

Results

Preliminary studies showed that the maximum dose of HC-3 which could be used without lowering the blood pressure was 500 μg./kg. but it was necessary to stimulate the nerve at a frequency of 1 or 2/sec. to produce an effect on the muscle response. Before investigating these effects, however, it was established that the response of the muscle to nerve stimulation at these frequencies was constant.

The effects produced by 100, 250 and 500 μg./kg. of HC-3 on the response of the muscle to nerve stimulation at a frequency of 1/sec. are compared with control results in Fig. 1*a*. This shows that 100 μg./kg. failed to produce a significant effect after 3 hr. A depression of the muscle response was produced, however, by doses of 250 and 500 μg./kg. The onset of the depression was similar for both doses and the maximum

effect occurred in approximately 90 min. Recovery from the effects of these doses occurred in 180 and 230 min. respectively.

Fig. 1*b* shows how the muscle response was modified by increasing the frequency of nerve stimulation to 2/sec. In contrast to Fig. 1*a* 100 μg./kg. caused a depression of the muscle response. Increasing the dose to 250 and 500 μg./kg. produced a successively greater depression. The rate of onset was similar for each dose but the times required to produce the maximum effects were 50, 70 and 80 min. respectively. Recovery from the effects of these doses of HC-3 occurred in 180 min. after 100 and 250 μg./kg. and in 280 min. following 500 μg./kg.

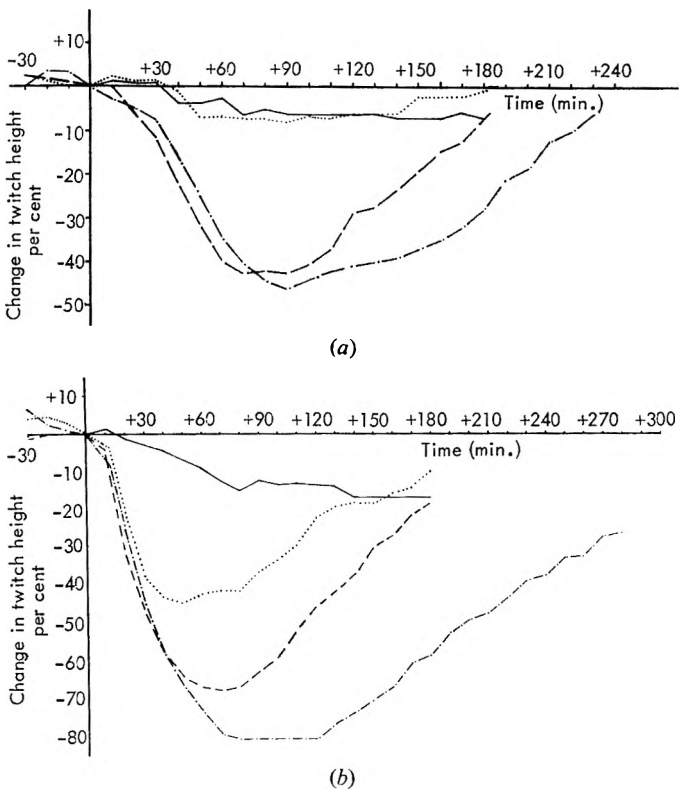

FIG. 1. The mean percentage change in twitch height produced on the cat tibialis anterior muscle stimulated by its nerve at a frequency of (*a*) 1/sec., (*b*) 2/sec. after intravenous injections of saline (——) and HC-3 in doses of 100 μg./kg. (. . . .), 250 μg./kg. (– – – –) and 500 μg./kg. (–·–·–·).

Point of intersection of the co-ordinates represents the point of drug administration.

Further experiments showed that when the response of the muscle to nerve stimulation was reduced by HC-3, the muscle was fully responsive to direct stimulation. This finding together with the observation that HC-3 failed to influence nerve conduction (Longo, 1959) indicates that

the effects described above are due to an action at the neuromuscular junction.

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The paper was presented by DR. EVANS.

MYASTHENIC-LIKE FEATURES OF THE NEUROMUSCULAR TRANSMISSION FAILURE PRODUCED BY TRIETHYLCHOLINE

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Received May 22, 1962

THE triethyl analogue of choline (triethyl-2-hydroxyethylammonium) has been shown to produce a slowly-developing failure of transmission in frequently excited mammalian nerve-muscle preparations. The cause of this, it has been suggested, is an action on the nerve endings through which the amount of acetylcholine released by a nerve impulse is reduced (Bowman and Rand, 1961a,b,c). Among a series of choline-analogues studied, optimal activity in this respect was found with the triethyl analogue (Bowman and Rand, 1962). In conscious animals, injection of triethylcholine causes a slowly developing muscular weakness which is accentuated by exercise and which closely resembles the symptoms of myasthenia gravis (Bowman and Rand, 1961a,b). However, preliminary observations appeared to suggest a difference between the defect in myasthenia gravis and that produced by triethylcholine. In the myasthenic patient, edrophonium and neostigmine cause a striking improvement in muscular power, but even when doses twice as great as the usual anti-curare doses were employed, these agents possessed only an insignificant ability to restore maximal twitches depressed by triethylcholine (Bowman and Rand, 1961b). The effects of larger doses of edrophonium and neostigmine on the muscular weakness produced by triethylcholine have now been examined both in conscious rabbits and in nerve-muscle preparations of anaesthetised cats.

Triethylcholine chloride (15 mg./kg.) was injected intravenously into each of 5 conscious rabbits. Every 5 min. after injection the righting-reflex was tested up to 20 times in rapid succession and the trial at which each rabbit failed to right itself was noted. The rabbits were left undisturbed between each test. Between 25 and 40 min. after injection, the rabbits lost the strength to right themselves after 2–10 trials and this degree of weakness lasted for about 20 min. A gradual recovery then occurred so that by 100 min. after injection they again responded to all 20 trials. Control tests previously carried out on the same rabbits showed that in the absence of drug treatment all of them continued to right themselves to all 20 trials applied at 5 min. intervals for up to 150 min., although their movements became less vigorous towards the end of this period.

On subsequent different days, the same doses of triethylcholine were administered and at the height of the ensuing muscular weakness, edrophonium (1 mg./kg.), neostigmine (0·25 mg./kg.) or choline (5 mg./kg.) was injected intravenously: the rabbits were atropinised (3 mg./kg. i.v.) before neostigmine or choline was administered. Edrophonium caused

a striking but temporary improvement in muscular strength, and neostigmine caused a slightly greater and longer lasting improvement which merged with the spontaneous recovery. However, neither of these was as effective as choline which caused a return almost to normal activity within 5–10 min. These results are expressed graphically in Fig. 1.

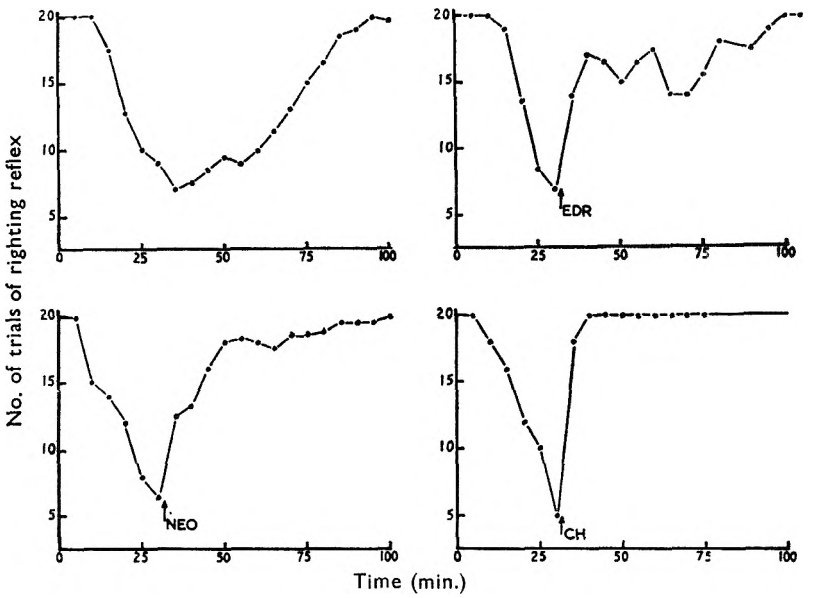

FIG. 1. The effects of edrophonium (EDR), neostigmine (NEO) and choline (CH) on the muscular weakness produced by triethylcholine in conscious rabbits. Each point represents the mean results of trials in 5 rabbits. Triethylcholine (15 mg./kg.) was injected intravenously at time 0 in each case. No antagonist was administered in the first experiment. For further details, see text.

In experiments on cats under chloralose anaesthesia the tension of the tibialis anterior muscle was recorded isometrically on a cathode ray oscilloscope by means of a RCA 5734 transducer valve; gross muscle action potentials were simultaneously led off by means of belly-tendon electrodes. Clonic contractions of the muscle were elicited by stimulation of the motor nerve with supramaximal shocks of 100 μsec. duration at a frequency of 10/sec. for 1 sec. every 10 sec. Fig. 2a is one of a series of identical clonic contractions recorded before triethylcholine. Between *a* and *b* a large dose of triethylcholine (100 mg./kg.) was injected intravenously. In order to hasten the onset of transmission failure, 12 tetani, each of 1 sec. duration and 100/sec. frequency, were delivered within the space of 1 min. after 10 min. had elapsed since the injection. In the normal muscle such high-frequency stimulation did not cause appreciable fatigue but after triethylcholine, the subsequent test responses were markedly depressed (Fig. 2b). This degree of transmission failure was maintained at a constant level for about 90 min. providing the stimulation was delivered at regular intervals throughout. After this period a gradual

recovery of the contractions occurred. Recovery could be hastened by allowing the preparation short periods of rest. Intravenous injections of edrophonium (0·5 mg./kg.), neostigmine (0·25 mg./kg.) or choline (5 mg./kg.) reversed the transmission failure. Fig. 2c illustrates the partial improvement in contractions produced by edrophonium. Twenty min. later the effect of edrophonium had worn off and the contractions had again become constant at a depressed level (Fig. 2d). Neostigmine produced about the same degree of antagonism as edrophonium but its duration of action was considerably longer. An injection of choline caused complete restoration of contractions (Fig. 2e) and this effect was persistent.

These results therefore show that, as in myasthenia gravis, edrophonium and neostigmine cause a marked improvement in the strength of muscles depressed by triethylcholine. Comparison of Fig. 2b and c with Fig. 6 of a paper by Desmedt (1961) illustrates the striking resemblance to the picture obtained with neostigmine in a similar experiment on a myasthenic patient.

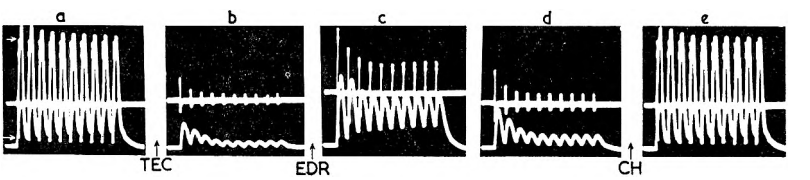

FIG. 2. Cat, chloralose anaesthesia. Isometric myogram (lower trace) and gross electromyogram (upper trace) recorded simultaneously on a cathode ray oscilloscope. The small horizontal arrows in 'a' indicate the levels of the peaks of the negative and positive deflections of the muscle action potentials which can be distinguished as small bulges in the tension trace. The gain of the action potentials was doubled in b, c and d. Between a and b, triethylcholine, between b and c, edrophonium and between d and e, choline was injected intravenously. c was recorded 5 min. after edrophonium and e was recorded 10 min. after choline. For further details see text.

A study of the literature makes it clear that both pre-junctional (Desmedt, 1960; Dahlbäck and others, 1961) and post-junctional defects (Churchill-Davidson and Richardson, 1953) may exist in generalised myasthenia gravis. There is also evidence (Stricker and others, 1960) that the defect is caused by a substance present in the blood stream and many extensive searches have been made for such an agent (e.g. Wilson, Obrist and Wilson, 1953; Wilson and Wilson, 1955; Nowell and Wilson, 1961; Nastuk and Strauss, 1961). If a circulating agent does exist, it might well possess properties like those of triethylcholine and there is evidence that an ethonium compound is normally present in nervous tissue. Thus Lorenté de No (1949), while working on the effects of quaternary ammonium ions on conduction in frog nerve fibres, extracted a quaternary compound with the properties of an ethonium ion from ox brain. More recently, Calvey, Nowell and Wilson (personal communication) found in chromatographic studies that a substance with an R_F value like that of the ethylcholines was present in the thymus glands of myasthenic patients and of foetal whales. An abnormal excess of

such a substance, possibly arising through some disfunction in choline metabolism, might therefore be the agent responsible for the defect in myasthenia gravis. Triethylcholine is not highly active in producing transmission failure in the experimental animal but under these circumstances it has to compete with the large amounts of choline normally present. Many choline analogues are acetylated by choline acetylase (Burgen and others, 1956), and it is possible that in exercise, when the traffic of nerve impulses is high, the motor nerve endings accept some choline analogue in place of choline and so release an inactive transmitter. The continual bombardment of the motor end-plates by such a false transmitter might then induce the post-junctional changes described by Churchill-Davidson and Richardson (1953).

Our experiments with triethylcholine have shown that choline is the best antagonist to its blocking action, but as yet there is no evidence that choline has any beneficial effects in myasthenia gravis. Acute treatment with choline might not produce a striking improvement because there is no reason to suppose that the post-junctional defect in myasthenics would immediately revert to normal. Prolonged treatment with choline, however, seems worthy of a trial.

Acknowledgements. One of us (W.C.B.) is grateful to the Central Research Fund of the University of London for a grant towards apparatus. B.A.H. is indebted to the Pharmaceutical Society of Great Britain for financial support.

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Discussion

The paper was presented by DR. BOWMAN. The following points were made in the discussion.

Twenty-five simple analogues of choline were examined, of which the best was triethylcholine. Some bis-quaternary compounds had also been

studied. Those with methyl groups attached to the quaternary nitrogen possessed a depolarising action, while those with large groups on the quaternary nitrogen had a curare-like action; compounds with groups intermediate in size behaved like triethylcholine. Chronic toxicity studies were made on triethylcholine in dogs, cats and rabbits, and no cumulative paralysing action was produced. The animals were not tested to find if the muscles had become truly myasthenic.

Short Communication

THE RESPONSE OF THE PIG UTERUS TO OXYTOCIN AT DIFFERENT STAGES IN THE OESTRUS CYCLE

By A. Knifton

From the Department of Pharmacology, University of Liverpool

Received May 23, 1962

Knaus (1926) showed that the uterus of the pregnant rabbit was insensitive to doses of Pituitrin which were sufficient to elicit large contractions in the non-pregnant state. This effect was later shown to be due to the influence of progesterone. But this difference in response between pregnant and non-pregnant uteri did not hold for some other species; thus in the rat and guinea-pig, the uterus responds to posterior pituitary extract (Pituitrin) in the gravid and non-gravid state. The evidence is reviewed by Reynolds (1949).

Adams (1940) tentatively suggested that in the sow, progesterone increases the sensitivity of the myometrium to posterior pituitary extract (Pituitrin). The present investigation was carried out in order to examine further this apparent anomaly.

Materials and Methods

Fresh pig uteri were collected from a nearby abattoir. A longitudinal strip was cut from one horn of each uterus, a circular strip from the cervix, and each strip suspended in a 10 ml. tissue bath containing oxygenated Tyrode solution at 39°. Each strip was connected by a thread to a lever to give a 10 × magnification of the contractions recorded on a smoked drum.

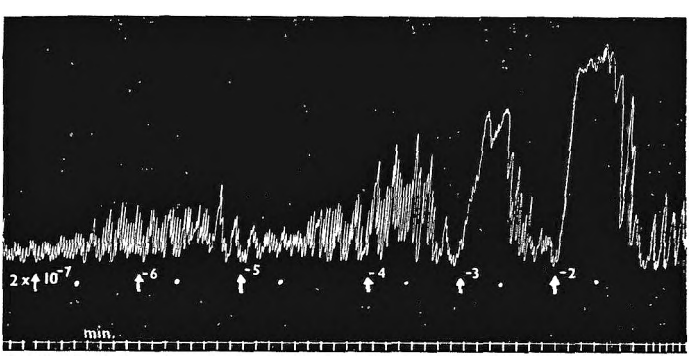

Fig. 1. Responses to oxytocin of a logitudinal strip from the body of a uterus in the luteal phase of the oestrus cycle. Oxytocin (units) was added to a 10 ml. bath at the times indicated by the arrows and washed out at the dots. The last three doses of oxytocin produced a rise in the base line, and this was therefore scored as a 50 per cent response.

After setting up each preparation, 1½ hr. was allowed to elapse for the uterine contractions to become regular before any drug was added to the bath. Oxytocin B.P. (Pitocin, Parke-Davis) was then added in increasing concentrations from 2×10^{-7} to 2×10^{-2} so that each uterus was tested with six different concentrations of drug (Fig. 1).

The response of each uterus was recorded as the number of times that a rise in the base line or an increased frequency of contraction was observed after the addition of each concentration of drug to the bath; this was scored as a percentage response. The stage in the oestrus cycle of each uterus was assessed by Corner's method (1921) which is based on histological examination of the uterus and corpora lutea. It was possible in this way to compare the response of uteri to oxytocin at various stages of the oestrus cycle and of pregnancy.

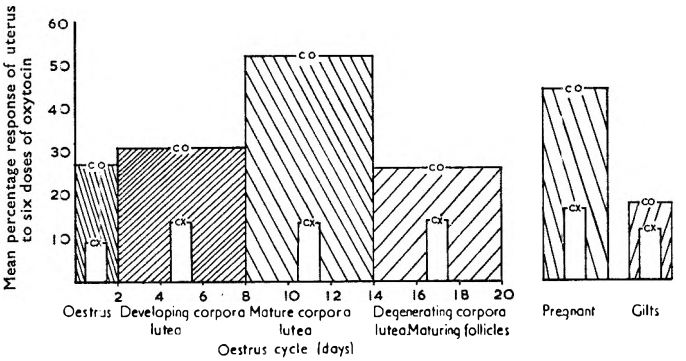

FIG. 2. The response of the cervix (cx) and body (co) of the pig uterus to oxytocin at different stages of the oestrus cycle and in pregnancy.

RESULTS AND DISCUSSION

Fifty-three sow uteri were examined, 50 at various stages of the oestrus cycle and 3 in early pregnancy; 15 uteri from immature gilts were also studied. The results (Fig. 2) show that uteri taken from animals between the 8th and 14th day of the oestrus cycle were the most sensitive. Since this is the phase at which the uterus is under the full influence of progesterone, it is clear that the sow uterus is more sensitive in the presence of this hormone than when under the influence of oestrogens. This is further supported by the evidence that uteri of pregnant sows were more sensitive than those of immature gilts in which the corpus luteum had not yet developed. In all cases the response of the body of the uterus was more sensitive than that of the cervix.

These results are in general agreement with those reported by Adams who used Pituitrin—presumably an extract of posterior pituitary gland containing both oxytocin and vasopressin. It is clear therefore that the response of the pig uterus, unlike that of the rabbit is enhanced in the presence of progesterone. This is probably an example of species difference in response to drugs. An important point to establish is whether the pig uterus under the influence of progesterone responds more effectively to oxytocin than to vasopressin and further work is in progress to investigate this using synthetic oxytocin and vasopressin.

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The paper was presented by THE AUTHOR.

AN APPARATUS FOR TESTING ANTICONVULSANT DRUGS BY ELECTROSHOCK SEIZURES IN MICE

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From The Distillers Company (Biochemicals) Limited, Fleming Road, Speke, Liverpool 24

Received May 21, 1962

An apparatus is described for assessing anticonvulsant drugs by the maximal electroshock seizure test in mice. It uses simple equipment which is normally available in pharmacological laboratories. The evaluation of three known and established anticonvulsant drugs show its application to routine testing procedures.

For the evaluation of anticonvulsant drugs the maximal electroshock seizure test described by Swinyard, Brown and Goodman (1952) has become well established. The apparatus described by Woodbury and Davenport (1952) is commonly used, a shock being administered to rats or mice through Spiegel (1937) electrodes applied to the eyes. The technique is time consuming and the stimulator output, which can reach 2,000 V, presents a potential hazard to the operator.

We describe here a simple application of the Palmer Electronic Square Wave Stimulator which has a maximum output of 100 V. Shocks were applied through ear electrodes.

Apparatus

The apparatus consists of an electronic square wave stimulator, providing an output easily adjustable for voltage, pulse rate and pulse width. The output is connected through a relay (2,000 ohms coil resistance) to silver electrodes. These were constructed from 0·04 in. diameter silver wire, sealed into glass tubes with the projecting ends fused to produce spherical tips. The relay controls the duration of the stimulus and is actuated photoelectrically through a rotating segment breaking a light beam focussed onto a phototransistor (Mullard OCP71). A segment of cardboard fixed to a kymograph provides a convenient rotating unit whereby the duration of the stimulus can be controlled by varying the speed of rotation of the kymograph and altering the size of the segment. The wiring diagram is shown in Fig. 1. For our experimental work a stimulus duration of 0·3 sec. was found to be satisfactory and this was obtained from a 90° segment rotating at 50 r.p.m.

Experimental

Methods

For the estimation of anticonvulsant activity, white mice (Schofield), weighing between 19 and 23 g., were used. Each mouse was only used once. The drugs compared were phenytoin, primidone and trimethadione, which were tested at the time of peak activity after dosing. They were administered orally, in aqueous solution, or suspended in mucilage

of tragacanth, to groups of mice at ascending dose levels in a constant volume of 0·5 ml. For administration of the shock the ears were filled with physiological saline and the mouse held with the electrodes placed in the ears. Whilst observing the rotating segment the impulse was switched on at the stimulator so that the shock was administered only once and for 0·3 sec.

From the numbers in each group showing tonic extensor convulsions, the ED50 values and confidence limits (P = 0·95) were calculated for each drug by the method of Litchfield and Wilcoxon (1949).

In order that protective indices could be calculated, the drugs were also tested for inco-ordination by the rotating rod method of Gross, Tripod and Meier (1955). The drugs were administered orally to groups of ten mice as before, and at hourly intervals after dosing they were placed individually on a $\frac{3}{4}$ in. metal rod rotating at 3 r.p.m. From the maximum number in each group failing to remain on the rod for 30 sec., the ID50 values and their confidence limits were calculated.

The ratio of the ID50 to the ED50 gives the protective index. Drugs effective clinically in grand mal epilepsy generally have a PI considerably greater than 1·0 in this test.

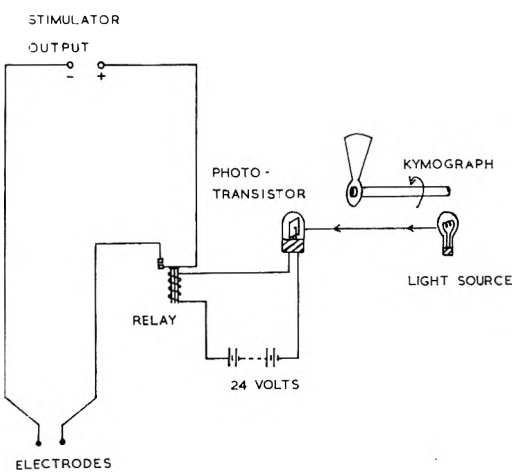

FIG. 1. Diagram showing arrangement of apparatus used in the maximal electroshock seizure test in mice.

RESULTS AND DISCUSSION

Stimulus

The stimulator output required for inducing the hind limb tonic extensor component of the maximal electroshock seizure pattern was first determined. Groups of ten mice were subjected to ascending stimuli by increasing the voltage and/or pulse width at the stimulator. Some indication of the current flowing was obtained by connecting an Avometer across the electrodes with the relay closed. The results (Table I) indicate that a supra-maximal effect was obtained at 80 V using a pulse rate of

100/sec. with a pulse width of 3 msec. for 0·3 sec. The recorded current output was 117 mA which was 2·5 times the current required to induce tonic extensor convulsions in 50 per cent of normal mice.

TABLE I

THE EFFECT OF INCREASING STIMULI ON TONIC EXTENSOR CONVULSIONS IN MICE

Stimulus		Recorded current, mA	Percentage of mice showing tonic extensor convulsions
Volts	Pulse width, msec.		
40	1	15	0
60	1	25·2	0
60	2	35	30
60	3	58	70
70	2	73	70
70	3	84	80
70	4	92	100
80	1·5	100	90
80	2	112	90
80	3	117	100
80	4	120	100
90	3	122	100

The pulse rate was 100/sec. and the duration of stimulation 0·3 sec. in all experiments.

Relative Activity of Drugs

The time of peak activity after dosing of each drug was determined in preliminary experiments. Groups of ten mice were dosed orally with each drug, the dose being the approximate ED50 as determined in a pilot test. At 1, 1½, 2, 2½, 3 and 4 hr. after dosing, groups of mice were subjected to the standard shock procedure and the numbers failing to exhibit tonic extensor convulsions were recorded. The times of peak activity were for phenytoin 2½ hr., primidone 3 hr. and trimethadione 1½ hr. respectively.

TABLE II

ANTICONVULSANT AND INCO-ORDINATION ACTIVITIES IN MICE

Drug	PAT* (hr.)	MES Test† ED50 mg./kg. orally	Inco-ordination Test TD50 mg./kg. orally	PI‡ (TD50/ED50)
Diphenylhydantoin ..	2½	5·7 (4·56 to 7·11)	36·3 (27·5 to 53·8)	6·37
Primidone	3	10·4 (3·9 to 19·5)	73·0 (33·1 to 161·1)	7·02
Trimethadione	1½	801·5 (622·7 to 1031)	420(267·6 to 659·1)	0·524

Figures in parenthesis indicate fiducial limits of error (P = 0·95).

* PAT = Peak activity time.
† MES = Maximum electroseizure test.
‡ PI = Protective index.

A comparison of the relative anticonvulsant activities (ED50) of the three drugs at their peak activity times in the maximum electroshock seizure test is shown in Table II, together with the results of the inco-ordination test (TD50) and the calculated protective indices.

The results show phenytoin to be the most potent drug both in the maximum electroshock seizure and the inco-ordination tests. The protective index was 6·37. Primidone was less active in both tests but

the protective index of 7·02 was very similar to that of phenytoin. Trimethadione showed a low activity in both tests and produced incoordination at a dose well below its anticonvulsant dose level, giving an index of 0·52. This drug is not used clinically in grand mal epilepsy.

These results are largely in accord with those reported in the literature for similar tests, with the exception of the ID50 for primidone, where Goodman and others (1953) found an ID50 in mice of 1,120 mg./kg. as opposed to the 73 mg./kg. we obtained. This discrepancy may be due to the difference in the method for determining neurological deficit or possibly the use by Goodman and his colleagues of a lower dose volume of this sparingly soluble compound. Their figure is not supported by the much higher toxicity they reported in cats, rabbits and humans.

Acknowledgment. Our thanks are due to Dr. G. F. Somers for advice and help in preparing the script.

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The paper was presented by MR. CASHIN.

LOCAL ANAESTHETIC ACTIVITY IN DIETHYLAMINOACETYL DERIVATIVES OF SUBSTITUTED BENZYLAMINES

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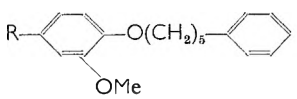
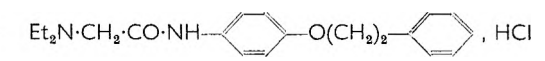
Six diethylaminoacetyl derivatives of general formula (III) are more potent than lignocaine as local anaesthetics. All cause erythema or necrosis of guinea-pig skin on intradermal injection except III, R = NH_2, R′ = H, and R″ = $O(CH_2)_2Ph$. This compound is several times more potent than lignocaine and in equipotent concentrations did not cause any tissue damage to skin on intradermal injection.

Local anaesthetic activity was first discovered by us in several aminophenoxyalkanes (Collins, Davis, Edge, Hill, Reading and Turnbull, 1959), including the primary amine (*Ia*) and its diethylamino-acetyl derivative (*Ib*). None of these compounds was as active as lignocaine and all caused erythema or necrosis of the skin after intradermal injection. A subsequent publication by Borovansky, Sekera and Vrba (1960) reported local anaesthetic activity in a compound (II) of this type but, although we



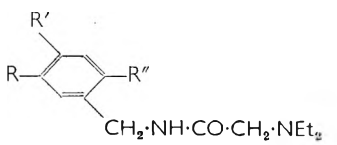
(*Ia*) R = NH_2, $MeSO_3H$
(*Ib*) R = $NH\,CO{\cdot}CH_2{\cdot}NEt_2$, HCl



(II)

found it to be more active than lignocaine, this compound also caused erythema and necrosis of guinea-pig skin on intradermal injection.

A claim by Dalal and Trivedi (1960), that diethylaminoacetyl derivatives of substituted benzylamines possessed local anaesthetic activity, led us to investigate a small group of related compounds (III) listed in Table I. Here we found much greater local anaesthetic activity, but tissue damage usually resulted from injection into the skin. One member of this series (III, R = NH_2, R′ = H, R″ = $O(CH_2)_2Ph$) (compound 8315), was several times as active as lignocaine in the tests described, and did not cause damage to the skin after intradermal injection of an equipotent concentration. In this respect it was the best compound examined.



(III)

Pharmacological Methods

The compounds were examined for local anaesthetic activity by four tests.

(1) *Surface Anaesthesia: Corneal Reflex Test of the Guinea-pig*

Eight albino guinea-pigs of either sex, weighing 400 to 500 g. were used for each compound (four animals on each of two concentrations). Concentrations, in normal saline from 1 to 2 per cent w/v for lignocaine and, for example, 0·005 to 0·02 per cent w/v for compound 8554, were continuously instilled into the conjuctival sac for 5 min. after which the eye was washed with 0·9 per cent w/v saline. The corneal reflex was tested by lightly touching the cornea with a rabbit's whisker six times at 5 min. intervals for 30 min., and the degree of anaesthesia was estimated by counting the times that the blink reflex could not be obtained during this period. For example, a score of 27 failures out of a possible maximum of 36 gave 75 per cent anaesthesia. Percentage anaesthesia was plotted against log concentration and relative potencies were abstracted by reading from the abscissa the concentration of each compound which produced 50 per cent anaesthesia.

(2) *Plexus Anaesthesia in Frogs* (Bülbring and Wadja, 1945)

Using 16 frogs the time for onset of anaesthesia was estimated for 3 concentrations of lignocaine (0·1, 0·2 and 0·5 per cent w/v in saline). Except for compounds 9329 and 9393 where three concentrations were used, each compound was then examined at two concentrations, these being chosen to give times within the range of the lignocaine results; 4 frogs were used at each concentration. Time for onset of anaesthesia was plotted against log concentration.

(3) *Infiltration Anaesthesia: Subcutaneous Injection into the Mouse Tail* (Bianchi, 1956)

Two per cent w/v solutions of each compound were prepared in distilled water and dilutions were made in 0·9 per cent w/v saline. Ten mice were used at each of 3 doses for each compound. Subcutaneous injections of 0·1 ml. were made in the tail about 1 cm. from the root and an artery clip applied to the tail at 15 min. intervals after injection up to 4 hr. A positive response occurred, usually within 5 sec., when a mouse attempted to remove the clip. A negative response was recorded, indicating local anaesthesia, when the mouse failed to attempt to dislodge the clip within 30 sec. The number of mice, out of ten, which gave a negative response was converted to percentage anaesthesia. Since maximal effects were observed 15 min. after injection, percentage anaesthesia at this time was plotted against log concentration. A comparison of the duration of action was afforded by extending the observations up to 4 hr. after injection.

(4) *Infiltration Anaesthesia: Intradermal Injection into the Skin of the Guinea-pig*

This method was based on that of Bülbring and Wadja (1945), as modified by Somers and Edge (1947). The compounds were injected intracutaneously in Latin square designs such that 9 fully grown albino guinea-pigs were used when comparing any compound with lignocaine

at 3 different concentrations. Graphs were plotted relating log concentration of the anaesthetic to the degree of anaesthesia 30 min. after injection. Since the lines obtained for all compounds were approximately parallel, relative potencies were calculated as the antilog of the differences in concentration between each compound and lignocaine in producing the same degree of anaesthesia.

Toxicity

Local toxicity to the skin was estimated by two methods.

(*a*) Observations were made on the mice used in test No. 3 for local anaesthetic activity, and a numerical score was allotted to each according to the severity of the local reaction. Three grades were easily distinguished ranging from erythema to severe necrosis. Daily observations were made up to 7 days, the maximum effect being usually observed after 4 days.

(*b*) The compounds were injected intradermally in a volume of 0·2 ml. into the shaved backs of guinea-pigs. Two concentrations of each compound were chosen, these were equipotent in local anaesthetic activity in test No. 4, with two concentrations of lignocaine (0·5 and 1·0 per cent). Each concentration was injected once into 4 guinea-pigs which were examined at intervals up to 7 days and the degree of irritation noted; six grades could be detected varying from slight erythema to severe necrosis. Confirmatory tests were made by histological examination of the skin and subcutaneous tissue.

Toxicity in mice. Albino mice of either sex weighing between 15 and 20 g. were injected intravenously and subcutaneously with the compounds under test, 30 to 40 mice being used for each LD50 determination. Observations were made up to 4 days after injection, though deaths usually occurred within 24 hr. of injection.

Pharmacological Results

All the results are summarised in Tables I and II.

Local Anaesthetic Activity

Test 1. Two experiments were carried out. In one, the figures were: lignocaine 1·0, compounds 7663, 0·0086, 8112, 0·10, 8315, 0·25 and 8554, 0·0044 per cent; in the second the figures were: lignocaine 1·78, compounds 9329, 0·02 and 9393, 0·08 per cent. The most potent compound was compound 8554 which was 224 times as effective as lignocaine.

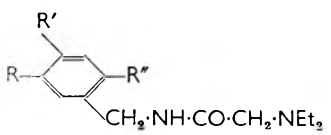
Test 2. One experiment was made for the estimation of conduction anaesthesia. With the exception of compound 8554, concentrations from 0·1 to 0·5 per cent w/v were used as this gave times for onset of anaesthesia similar to those for lignocaine in the same concentrations. The graphs when plotted were not parallel but the differences from lignocaine were so slight that the compounds have been accorded a figure approximately equal to lignocaine. Compound 9329 is quoted as less active because a linear relationship between log concentration

and effect did not exist; anaesthesia developed in about 20 min. regardless of concentration. Compound 8554 (0·05 per cent) gave a mean time to anaesthesia equal to that of lignocaine (0·5 per cent), thus showing a relative potency 10 times that of lignocaine.

Test 3. The compounds were tested in two groups in different experiments. In the first the concentrations producing 50 per cent anaesthesia were as follows: compounds 7663, 0·21, 8112, 0·34, 8315, 0·20 and 8554, 0·24 per cent, compared with 0·58 per cent for lignocaine. In the second

TABLE I

RELATIVE ANAESTHETIC POTENCIES AND TOXICITIES OF DIETHYLAMINOACETYL DERIVATIVES OF SUBSTITUTED BENZYLAMINES

General formula 

Compound No.	R	R′	R″	Test	Anaesthetic activity (approx.) lignocaine = 1	LD50 (mg./kg.) (mice) i.v.	LD50 (mg./kg.) (mice) s.c.
7663	NH_2	H	OC_8H_{17}	1 2 3 4	118 ≃1 3 10	8 (87–115)	—
8112	Et_2NCH_2CONH	H	OC_8H_{17}	1 2 3 4	10 ≃1 1·75 7	37·5 (92–109)	550 (87–115)
8315	NH_2	H	$O(CH_2)_2Ph$	1 2 3 4	4 ≃1 3 4	26 (92–109)	225 (77–130)
8554	H	H	OC_8H_{17}	1 2 3 4	224 10 2·3 47	5 (78–128)	290 (87–115)
9329	H	OC_8H_{17}	H	1 2 3 4	87 <1 0·6 ≃3	42 (80–125)	>2000 —
9393	H	H	$O(CH_2)_2Ph$	1 2 3 4	22 ≃1 0·8 5	14 (86–116)	>1000 —

All compounds were tested as the mono- or dihydrochlorides. The LD50 values for lignocaine hydrochloride were 15 mg./kg. (limits 87–115 per cent for P = 0·95) by i.v. injection and 390 mg./kg (limits 81–124 per cent) by s.c. injection. Figures in brackets under LD50 show the fiducial limits (per cent) for P = 0·95.

experiment the figures were: lignocaine 0·15, compounds 9329, 0·24 and 9393, 0·18 per cent. Lignocaine used as a standard had a duration of action about 45 min., compounds 8315 and 9393 about 1 hr., whereas the remainder had an action lasting 2 hr. or more.

Test 4. The relative activities shown in Table I were obtained from one experiment. Concentrations of lignocaine from 0·1 to 0·4 per cent were used and the concentrations of compound 8554, the most active compound in this test, ranged from 0·0025 to 0·01 per cent.

Effect of Adrenaline on Local Anaesthetic Action

One experiment was made to determine the effect of adrenaline on the anaesthetic activity of compound 8315. These results were obtained after 5 hr. by the guinea-pig intradermal test, and the results are shown in Table II. All dilutions of compound 8315 and lignocaine were made in 1 : 200,000 adrenaline hydrochloride solution. Adrenaline potentiated the local anaesthetic activity of compound 8315 to a greater degree than that of lignocaine (concentrations were used which were equipotent when compound 8315 and lignocaine were used alone without adrenaline.)

TABLE II

EFFECT OF ADRENALINE HYDROCHLORIDE ON LOCAL ANAESTHETIC ACTIVITY OF COMPOUND 8315, USING THE GUINEA-PIG INTRADERMAL WHEAL TEST

Compound	Concentration (per cent)	Percentage anaesthesia after 5 hr.	
		(*a*) In aqueous solution	(*b*) In 1 : 200,000 adrenaline hydrochloride solution
Lignocaine	1	42	54
	0·5	33	58
Compound 8315	1	75	96
	0·5	58	88
	0·25	50	83
	0·125	33	79

Toxicity

Local toxicity to the skin. All the compounds, with the exception of compound 8315, caused slight to severe necrosis of the skin, with an inflammatory cellular reaction in concentrations which were equipotent with lignocaine in producing anaesthesia by intradermal injection. Lignocaine produced but a slight inflammatory cellular infiltration at a concentration of 1 per cent. Compound 8315 at 0·25 per cent produced almost identical reactions and at 1 per cent gave rise to moderate necrosis of the skin and localised muscle degeneration.

Toxicity in mice. The LD50 figures for intravenous and subcutaneous injection are shown in Table I. Compound 8315 was less toxic than lignocaine on intravenous injection but more toxic on subcutaneous injection. All the compounds, including lignocaine, produced loss of the righting reflex with an increase in respiration rate; death was usually preceded by tonic convulsions except for lignocaine where no convulsions were observed.

DISCUSSION

The small series of diethylaminoacetyl derivatives of substituted benzylamines described in this paper showed interesting local anaesthetic properties, as determined by several tests in mice, guinea-pigs and frogs for infiltration, conduction and topical anaesthesia.

By topical application to the cornea of the guinea-pig all six compounds were considerably more potent than lignocaine. Compounds

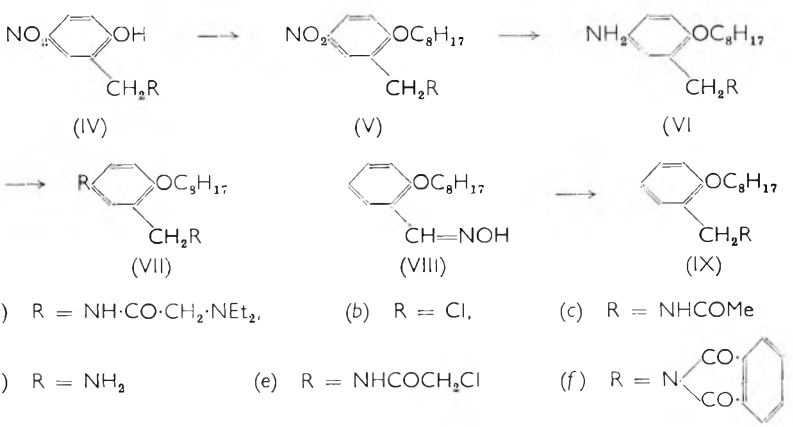
7663, 8554 and 9329 were 100 to 200 times as active as lignocaine but only in this test; by the other tests they were generally only 1 to 50 times as active.

In the small group of compounds tested all those with the OC_8H_{17} grouping produced a severe local reaction, on injection into guinea-pig skin, in concentrations which were equipotent (as local anaesthetics) with lignocaine. The only compound which was as well tolerated as lignocaine, namely 8315, possessed an $O(CH_2)_2Ph$ grouping. It should be noted however that one other compound with this grouping (9393) caused moderate necrosis.

It is difficult to explain why these compounds should show such high activity on the cornea. Pertinent to this point are some observations of relative surface tension by a capillary rise method: one per cent aqueous solutions showed the following order: lignocaine $>$ 8315 $>$ 7663, 8112, 8554, 9329 and 9393. Lowering of surface tension may play some part in permitting the compounds to penetrate more readily into the cornea than into other tissues.

Chemical Methods

Compound 7663 (VI*a*) was synthesised from the known 2-diethylamino-acetamidomethyl-4-nitrophenol (IV*a*) by alkylation to the n-octyl ether (V*a*) and subsequent catalytic reduction. In a second route, 2-chloro-



methyl-4-nitrophenol (IV*b*) was converted successively into 2-acetamido-methyl-4-nitrophenol (IV*c*) and its n-octyl ether (V*c*). Hydrolysis gave the primary amine (V*d*), which was chloroacetylated to (V*e*), treated with diethylamine, yielding (V*a*), and then reduced. A third route utilised 5-nitro-2-n-octyloxybenzyl chloride (V*b*) available from concurrent work. From this the 2-phthalimidomethyl compound (V*f*) was prepared and was hydrolysed to the amine (V*d*), subsequent stages being as already described. The nitro-amine (V*d*) was also used for the synthesis of compound 8112 (VII*a*) by reduction to the diamine (VI*d*), chloro-acetylation, and treatment of the product (VII*e*) with diethylamine.

The phenethyl ether 8315 was obtained from 2-acetamidomethyl-4-nitrophenol (IV*c*) by a route similar to the second one described above.

Compound 8554 was prepared from the octyl ether of salicylaldehyde via the oxime (VIII) followed by reduction to the amine (IX*d*), chloroacetylation, and reaction with diethylamine. Compound 9393 was similarly obtained from the corresponding phenethyl ether of salicylaldehyde, and 9329, the *para*-isomer of compound 8554, was likewise prepared from *p*-hydroxybenzaldehyde.

EXPERIMENTAL

1-(2-Diethylaminoacetamidomethyl-4-nitrophenoxy)-n-octane hydrochloride (V*a*). (*a*) 2-Diethylaminoacetamidomethyl-4-nitrophenol (IV*a*) (11 g.) (Einhorn and Mauermayer, 1905) was dissolved in a solution prepared from sodium (0·82 g.) and ethanol (30 ml.). n-Octyl bromide (7·5 g.) was added, the mixture was refluxed (12 hr.), ethanol was removed and the residue was extracted with ether. The ethereal solution was washed with N aqueous sodium hydroxide and water, dried and saturated with dry hydrogen chloride. The product was crystallised from ethanol giving (*1-(2-diethylaminoacetamidomethyl-4-nitrophenoxy)-n-octane hydrochloride* (3·5 g.), m.p. 168–170°. Found: N, 9·7; Cl, 8·5. $C_{21}H_{35}N_3O_4$, HCl requires N, 9·8; Cl, 8·3 per cent.

(*b*) 5-Nitro-2-n-octyloxybenzyl chloride (V*b*) (118 g.) (Collins, unpublished) in acetone (1 litre) was refluxed (0·5 hr.) with sodium iodide (59 g.): sodium chloride was then filtered off. A solution of potassium hydroxide (84 per cent, 25·4 g.) and phthalimide (93·5 g.) in water (200 ml.) was added and the mixture was refluxed (24 hr.) and cooled. The product was recrystallised from ethanol, giving *1-(4-nitro-2-phthalimidomethylphenoxy)-n-octane* (V*f*) (75 g.), m.p. 103–105°. Found: N, 6·8. $C_{23}H_{26}N_2O_5$ requires N, 6·8 per cent.

A solution of this compound (15 g.) in ethanol (50 ml.) and aqueous hydrazine (60 per cent w/v, 6·25 ml.) was refluxed (3 hr.), ethanol was removed and the residue was extracted with a mixture of concentrated hydrochloric acid (16 ml.) and water (26 ml.). The filtered solution was basified with aqueous sodium hydroxide and extracted with ether, yielding 1-(2-aminomethyl-4-nitrophenoxy)-n-octane (V*d*) (10 g.). A solution of this base (21 g.) in acetic acid (150 ml.) containing anhydrous sodium acetate (5 g.) was treated with chloroacetyl chloride (6·55 ml.). After 30 min. the mixture was poured into water and the product was recrystallised from aqueous acetic acid to yield *1-(2-chloroacetamidomethyl-4-nitrophenoxy)-n-octane* (V*e*) (10·4 .g), m.p. 85–88°. Found: N, 7·5; Cl, 9·3. $C_{17}H_{25}ClN_2O_4$ requires N, 7·8; Cl, 9·9 per cent. The chloroacetyl derivative (10·3 g.) was refluxed (3 hr.) with diethylamine (30 ml.) and the excess of diethylamine was removed. An ether solution of the residue was washed with water, dried and treated with hydrogen chloride, yielding 1-(2-diethylaminoacetamidomethyl-4-nitrophenoxy)-n-octane hydrochloride (7·6 g.), m.p. 168–171°, not depressed by a sample prepared as in (*a*).

(*c*) 2-Chloromethyl-4-nitrophenol (IV*b*) (300 g.) and acetamide (900 g.) were heated (1 hr.) at 170–180°. The mixture was cooled and poured into water to yield 2-*acetamidomethyl*-4-*nitrophenol* (IV*c*) (303 g.), m.p. 194–196° (from ethyl acetate). Found: C, 51·4; H, 5·0; N, 13·2. $C_9H_{10}N_2O_4$ requires C, 51·4; H, 4·8; N, 13·3 per cent.

This compound (147 g.) was dissolved in 2-ethoxyethanol (1 litre) and treated with sodium hydroxide (28 g.) in water (30 ml.) then n-octyl bromide (135 g.). After 8 hr. reflux the solution was diluted with water and the product was recrystallised from aqueous methanol to yield 1-(2-*acetamidomethyl*-4-*nitrophenoxy*)-*n*-*octane* (V*c*) (182 g.), m.p. 73–74°. Found: C, 63·6; H, 8·1; N, 8·6. $C_{17}H_{24}N_2O_3$ requires C, 63·6; H, 8·1; N, 8·7 per cent. A mixture of this compound (182 g.) with hydrochloric acid (200 ml.) and water (200 ml.) was refluxed (8 hr.) and the hydrochloride was collected. It was suspended in water and treated with excess of sodium hydroxide to yield an oil, 1-(2-aminomethyl-4-nitrophenoxy)-n-octane (V*d*) (169 g.). The remaining stages were as in (*b*).

1-(4-*Amino*-2-*diethylaminoacetamidomethylphenoxy*)-*n*-*octane* (compound 7663; VIa). The corresponding nitro-compound (11 g.) was hydrogenated at Adams's platinum oxide (220 mg.) in ethanol (200 ml.) at 60°/70 lb. per sq. in. The product was treated with ethereal hydrogen chloride to yield 1-(4-*amino*-2-*diethylaminoacetamidomethylphenoxy*)-*n*-*octane dihydrochloride* (9 g.), softens from 85°. Found: N, 9·6; Cl, 16·0. $C_{21}H_{37}N_3O_2$, 2HCl required N, 9·6; Cl, 16·3 per cent. The (−)-*di*-*p*-*toluoyltartrate* had m.p. 106–110°. Found: C, 65·7; H, 7·3; N, 5·0. $C_{21}H_{37}N_3O_2,C_{20}H_{18}O_8$ requires C, 65·7; H, 7·3; N, 5·6 per cent.

1-(4-*Diethylaminoacetamido*-2-*diethylaminoacetamidomethylphenoxy*)-*n*-*octane dihydrochloride* (compound 8112, VIIa). 1-(2-Aminomethyl-4-nitrophenoxy)-n-octane (Vd) (12 g.) was hydrogenated as above at 30°/200 lb. per sq. in. The hydrochloride of the resulting 1-(4-amino-2-aminomethylphenoxy)-n-octane (VId) was dissolved in acetic acid (100 ml.) containing anhydrous sodium acetate (14 g.). Chloroacetyl chloride (12 ml.) was added and the mixture was stirred (2 hr.) then poured into water. The crude product, which slowly solidified, was recrystallised from methanol to yield 1-(4-*chloroacetamido*-2-*chloroacetamidomethylphenoxy*)-n-*octane* (VIIe) (7·2 g.), m.p. 137–139°. Found: N, 7·0; Cl, 17·5. $C_{19}H_{26}Cl_2N_2O_3$ requires N, 7·0; Cl, 17·7 per cent. This compound (5·3 g.) was refluxed (3 hr.) with diethylamine (22 ml.), the excess of diethylamine was removed, the residue was treated with water and extracted with ether. The washed and dried ethereal solution was concentrated, and the base was converted with ethereal hydrogen chloride into 1-(4-*diethylaminoacetamido*-2-*diethylaminoacetamidomethylphenoxy*)-*n*-*octane dihydrochloride* (6·5 g.), m.p. 83–85°. Found: N, 10·2; Cl, 12·6. $C_{27}H_{48}N_4O_3$,2HCl requires N, 10·2; Cl, 12·9 per cent.

1-(4-*Amino*-2-*diethylaminoacetamidomethylphenoxy*)-2-*phenylethane dihydrochloride* (compound 8315). A solution of 2-acetamidomethyl-4-nitrophenol (IVc) (35 g.) and sodium hydroxide (6·7 g.) in 2-ethoxyethanol (240 ml.) and water (8 ml.) was refluxed (7 hr.) with 2-phenyl-

ethyl bromide (30·8 g.). The mixture was cooled and poured into water (240 ml.). The crude product was recrystallised from methanol yielding 1-(2-acetamidomethyl-4-nitrophenoxy)-2-phenylethane (24 g.), m.p. 139–141°. Hydrolysis of this compound (24 g.) was effected by refluxing (8 hr.) with a mixture of hydrochloric acid (50 ml.) and water (50 ml.). The hydrochloride which separated from the cooled mixture was suspended in water and basified with sodium hydroxide, giving 1-(2-aminomethyl-4-nitrophenoxy)-2-phenylethane (19 g.) as an oil. To the base in acetic acid (150 ml.) containing anhydrous sodium acetate (4·7 g.), chloroacetyl chloride (6 ml.) was added. The mixture was stirred (5 min.), kept 30 min., and treated with water (160 ml.). The product (10 g.), m.p. 103–105°, was recrystallised from benzene to yield 1-(2-*chloroacetamidomethyl*-4-*nitrophenoxy*)-2-*phenylethane* (5·4 g.), m.p. 117–119°. Found: N, 7·8; Cl, 10·3. $C_{17}H_{17}ClN_2O_4$ requires N, 8·0; Cl, 10·2 per cent.

The above chloroacetyl derivative (9·8 g.) was refluxed (3 hr.) with diethylamine and worked up in the usual way. The product was converted into the hydrochloride, which was recrystallised from ethanol to yield 1-(2-*diethylaminoacetamidomethyl*-4-*nitrophenoxy*)-2-*phenylethane hydrochloride* (8·7 g.), m.p. 215–217°. Found: N, 9·9; Cl, 8·6. $C_{21}H_{27}N_3O_4$,HCl requires N, 10·0; Cl, 8·4 per cent. Hydrogenation of this compound (8·65 g.) over Raney nickel in ethanol (150 ml.) at 57°/70 lb. per sq. in. gave the hygroscopic 1-(4-*amino*-2-*diethylamino-acetamidomethylphenoxy*)-2-*phenylethane hydrochloride* (6·0 g.). m.p. 138–142°. Found: N, 11·2; Cl, 8·7. $C_{21}H_{29}N_3O_2$,HCl requires N, 10·7; Cl, 9·0 per cent. It was converted into the *dihydrochloride*, m.p. 89–115° (efferv.). Found: N, 9·6; Cl, 16·1. $C_{21}H_{29}N_3O_2$,2HCl requires N, 9·8; Cl, 16·6 per cent.

1-(2-*Diethylaminoacetamidomethylphenoxy*)-*n-octane* (compound 8554; IXa). Redistilled salicylaldehyde (24·4 g.), n-octyl bromide (46 g.), ethanol (100 ml.) and anhydrous potassium carbonate (53 g.) were refluxed (18 hr.) with stirring. The solvent was removed and the residue was extracted with ether. The washed and dried extract was concentrated and the residue was distilled to yield o-*octyloxybenzaldehyde** (40·2 g.), b.p. 130–133°/0·02 mm. n_D^{20} 1·5118. Found: C, 76·7; H, 9·4. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5 per cent. The aldehyde (13 g.) in ethanol (100 ml.) was mixed with hydroxylamine hydrochloride (4 g.) in water (20 ml.) and potassium hydroxide (4 g.) in water (10 ml.). The mixture was refluxed (30 min.) and poured into water. The product was dried and recrystallised from ethanol or light petroleum (b.p. 40–60°) to yield o-*octyloxybenzaldoxime* (VIII) (7·4 g.), m.p. 33–36°. Found: N, 5·4. $C_{15}H_{23}NO_2$ requires N, 5·6 per cent.

The oxime (10 g.) in dry ethanol (100 ml.) was reduced with powdered sodium (10 g.) added in portions. Solvent was removed, water was added, and the amine was taken up in ether. The extract was shaken with 2N aqueous hydrochloric acid, the acid solution was basified and the

* Prepared by Dr. D. A. A. Kidd of these laboratories.

amine was re-extracted into ether. The dried ethereal solution was concentrated and distilled giving o-*octyloxybenzylamine* (IXd) (4·9 g.), b.p. 156°/0·02 mm. n_D^{20} 1·5049. This amine (4·8 g.), in acetic acid (50 ml.) containing anhydrous sodium acetate (1·7 g.), was treated with chloroacetyl chloride (2·4 g.) as before. The mixture was diluted with water and the product was dried and recrystallised from light petroleum (b.p. 40–60°) to yield crude 1-(2-chloroacetamidomethylphenoxy)-n-octane (IXe) (5·5 g.), m.p. 49–50°. Found: N, 4·6; Cl, 10·0. $C_{17}H_{26}ClNO$ requires N, 4·5; Cl, 11·4 per cent. The low chlorine analysis was due to contamination with the corresponding acetyl derivative.

The crude chloroacetyl derivative (5·4 g.) was treated with diethylamine in the usual way. The hydrochloride obtained was dissolved in water and extracted with ether to remove non-basic material (the acetyl derivative) and the aqueous solution was then basified and extracted with ether. Distillation gave 1-(2-*diethylaminoacetamidomethylphenoxy*)-*n-octane* (2·8 g.), b.p. 175–180°/0·04 mm. Found; C, 72·1; H, 10·5; N, 7·9. $C_{21}H_{36}N_2O_2$ requires C, 72·4; H, 10·4; N, 8·0 per cent. The hydrochloride was obtained as a pale yellow gum which could not be crystallised.

1-(2-*Diethylaminoacetamidomethylphenoxy*)-2-*phenylethane* (compound 9393). This compound was similarly prepared from salicylaldehyde. The following intermediates were obtained (yields are given in parentheses): o-(2-*Phenylethoxy*)*benzaldehyde* (57 per cent), m.p. 71–73·5° (from ethanol). Found: C, 78·4; H, 6·3. $C_{15}H_{14}O_2$ requires C, 79·5; H, 6·2 per cent. o-(2-*Phenylethoxy*)*benzaldoxime* (95 per cent), m.p. 90–92° [from light petroleum (b.p. 80–100°)]. Found: C, 74·6; H, 6·5; N, 6·0. $C_{15}H_{15}NO_2$ requires C, 74·7; H, 6·2; N, 5·8 per cent. o-(2-*Phenylethoxy*)*benzylamine* (50 per cent), b.p. 148–155°/0·05 mm. Found: C, 79·5; H, 7·7; N, 6·0. $C_{15}H_{17}NO$ requires C, 79·2; H, 7·5; N, 6·2 per cent. 1-(2-*Chloroacetamidomethylphenoxy*)-2-*phenylethane* (71 per cent), m.p. 96–98° [from light petroleum (b.p. 80–100°)]. Found: N, 4·6; Cl, 11·5. $C_{17}H_{18}ClNO_2$ requires N, 4·6; Cl, 11·7 per cent. 1-(2-*Diethylaminoacetamidomethylphenoxy*)-2-*phenylethane* (57 per cent), b.p. 210–220°/0·1 mm. Found: C, 73·8; H, 8·5; N, 8·1. $C_{21}H_{28}N_2O_2$ requires C, 74·0; H, 8·2; N, 8·2 per cent. The hydrochloride was a yellow gum.

1-(4-*Diethylaminoacetamidomethylphenoxy*)-*n-octane* (compound 9329). This compound was similarly prepared from *p*-hydroxybenzaldehyde. The following intermediates were obtained: p-*Octyloxybenzaldehyde* (83 per cent), b.p. 134–144°/0·1 mm. Found: C, 76·3; H, 9·6. $C_{15}H_{22}O_2$ requires C, 77·0; H, 9·4 per cent. p-*Octyloxybenzaldoxime* (77·5 per cent), m.p. 81–83° (from aqueous ethanol). Found: N, 5·7. $C_{15}H_{23}NO_2$ requires N, 5·6 per cent. p-*Octyloxybenzylamine* (32 per cent), b.p. 130–140°/0·05 mm. Found: C, 76·4; H, 10·6; N, 5·8. $C_{15}H_{25}NO$ requires C, 76·5; H, 10·6; N, 5·9 per cent. 1-(4-*Chloroacetamidomethylphenoxy*)-*n-octane* (76 per cent), m.p. 97–99° (from aqueous acetic acid). Found: N, 4·75; Cl, 12·3. $C_{17}H_{26}ClNO_2$ requires N, 4·5; Cl, 11·5 per cent. 1-(4-*Diethylaminoacetamidomethylphenoxy*)-*n-octane* (48

per cent), m.p. 53–54° [from light petroleum (b.p. 40–60°)]. Found: C, 72·3; H, 10·3; N, 8·2. $C_{21}H_{36}N_2O_2$ requires C, 72·5; H, 10·3; N, 8·05 per cent.

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The paper was presented by MR. LARGE.

THE DETERMINATION OF ERGOTAMINE IN PREPARATIONS CONTAINING ERGOTAMINE TARTRATE AND CYCLIZINE HYDROCHLORIDE

By A. C. Caws and B. E. Lawrence

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Benzhydrol, an impurity in some samples of cyclizine hydrochloride, interferes with the colorimetric estimation of ergotamine in preparations containing ergotamine tartrate and cyclizine hydrochloride. A method for overcoming this difficulty is described. The inhibition of colour development has been utilised to provide a method for the detection and estimation of benzhydrol in cyclizine hydrochloride.

The use of ergotamine tartrate for the treatment of migraine has resulted in the clinical use of compound tablets containing, besides ergotamine tartrate, a stimulant such as caffeine or a drug to combat sickness or both. In this connection we became interested in the assay of granules, containing ergotamine tartrate and cyclizine hydrochloride. It was found that when powdered granules were shaken with 1 per cent tartaric acid the resulting extract could be assayed colorimetrically for ergotamine using dimethylaminobenzaldehyde solution B.P.: this procedure was adopted for routine work. Some batches of granules, however, gave unexpectedly low results for ergotamine content and we were satisfied that the ergotamine tartrate had been put into the granules and that the assay procedure was faulty. It was discovered that solutions of ergotamine tartrate and cyclizine hydrochloride in 1 per cent tartaric acid gave satisfactory assay results when freshly prepared, but underwent a gradual change when heated on a steam bath and after 30 min. gave no colour with the official reagent. Solutions of ergotamine tartrate alone were unaffected by this treatment. It was decided therefore to avoid heating during the assay of the granules but, despite this, low results for the ergotamine content were still occasionally obtained. We concluded that some samples of cyclizine hydrochloride contained an impurity which interfered with the colour reaction.

To test this, a solution of ergotamine tartrate in 1 per cent tartaric acid was divided into eight equal portions. To each was added the appropriate amount of cyclizine hydrochloride to afford a solution similar to that used in the assay of the granules, a different batch of cyclizine hydrochloride being used for each solution. All solutions were then assayed colorimetrically for ergotamine. The results showed clearly that some samples of cyclizine hydrochloride interfered with the colour reaction and some did not.

Attempts made to isolate the interfering substance by fractional crystallisation were unsuccessful. When, however, an aqueous solution of "impure" cyclizine hydrochloride was distilled at atmospheric pressure a cloudy distillate was obtained which, after standing for several days,

deposited colourless needles, of m.p. 65°. This material strongly interfered with the ergot colour reactions (Table I). It was identified as benzhydrol; its ultra-violet absorption curve was superimposable on that of an authentic specimen determined under identical conditions and it gave no depression in m.p. on admixture with an authentic specimen.

Once identified, the impurity was easily removed from the tartaric acid extract of the granules by ether extraction and the ergotamine was then quantitatively recovered during the assay.

TABLE I

INFLUENCE OF BENZHYDROL ON COLORIMETRIC ASSAY OF ERGOTAMINE TARTRATE

Weight of benzhydrol in 50 ml. of 1 per cent tartaric acid (mg.)	Per cent recovery of ergotamine tartrate by assay
—	100
0·31	90·3
0·77	70·9
1·08	60·0
1·51	49·3
2·11	38·2
2·32	37·2
3·09	30·3
4·22	24·3
7·24	20·2

Each assay was performed on a 0·00754 per cent w/v solution of ergotamine tartrate in aqueous 1 per cent tartaric acid solution.

This work provides the basis for a qualitative and quantitative test for the presence of benzhydrol in samples of cyclizine hydrochloride.

The following method was evolved for the determination of ergotamine in granules containing 87·7 per cent cyclizine hydrochloride and 3·5 per cent ergotamine tartrate.

Method

Transfer an accurately weighed quantity of about 0·4 g. of finely powdered granules to a 100 ml. graduated flask, add aqueous 10 per cent w/v tartaric acid solution (75 ml.) and shake mechanically for 30 min. Adjust the volume to 100 ml. with the tartaric acid solution, mix thoroughly and filter through a 9 cm. Whatman No. 1 filter paper rejecting the first 25 ml. of filtrate.

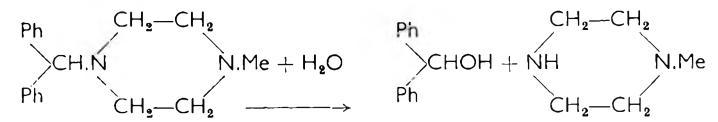
Transfer 50 ml. of the filtrate to a 100 ml. separating funnel, add ether (25 ml.) and shake thoroughly. Allow to stand for 5 min. and transfer the aqueous layer to a 100ml. graduated flask. Wash the ether layer with four successive 10 ml. portions of the tartaric acid solution adding the washings to the bulk of the solution in the 100 ml. flask. Adjust the contents of the flask to 100 ml. with the tartaric acid solution and mix. Transfer a 5 ml. aliquot of the solution to a 25 ml. flask and slowly add dimethylaminobenzaldehyde solution B.P. (10 ml.); mix thoroughly and set aside for 15 min. Determine the ergotamine tartrate content by comparing the extinction of this solution at 550 mμ with that obtained by treating a standard ergotamine tartrate solution in an identical manner.

Estimation of Benzhydrol in Cyclizine Hydrochloride

The inhibition of colour development in this reaction has been used as a test for benzhydrol in production batches of cyclizine hydrochloride. A calibration curve, constructed from data in Table I, has been used tentatively for quantitative estimation. Thus a sample which lowered the ergotamine recovery by 20 per cent was estimated to contain 0·45 per cent of benzhydrol. As a check measurement of the extinction at 225 mμ and 204 mμ of an ether extractive of this sample gave the benzhydrol content as 0·425 per cent. A second sample, which showed no significant inhibition of the colour reaction, was found to contain 0·009 per cent of benzhydrol by this criterion.

Discussion

All batches of cyclizine hydrochloride used, complied fully with the B.P.C. 1959 monograph. The isolation of benzhydrol led us to consider the possible source of this impurity. This can arise firstly by hydrolysis of diphenylmethyl halide used in manufacture or secondly by 'hydrolysis' of cyclizine in the following manner.



Cyclizine Benzhydrol *N*-Methylpiperazine

Pertinently, when cyclizine hydrochloride, free from interfering agent, was dissolved in water and the solution boiled under reflux for some hours, both benzhydrol and benzophenone could be recovered from the aqueous solution by distillation. The latter no doubt had been formed by oxidation of benzhydrol. Neither benzophenone nor methylpiperazine interfered with the ergot colour reaction.

Benzhydrol inhibits the colour reaction with lysergic acid in a similar manner and we have no doubt that it would do the same with other ergot alkaloids. Some observations have an interesting bearing on the mechanism whereby benzhydrol inhibits this colour reaction of ergotamine. A solution of ergotamine tartrate and cyclizine hydrochloride (giving a satisfactory colour reaction) gave practically no colour after heating on a steam bath for 30 min. The same treatment of ergotamine tartrate solution alone or of cyclizine hydrochloride alone, led to no such colour inhibition. This suggests that "hydrolysis" of cyclizine—normally slow, is accelerated in some way by the ergotamine. It was established that a solution of benzhydrol in 1 per cent tartaric acid gave no colour reaction with dimethylaminobenzaldehyde solution.

The tentative method has shown that while some samples of cyclizine hydrochloride contain benzhydrol as an impurity the maximum content corresponds to no more than 0·5 per cent.

Discussion

The paper was presented by Mr. Caws. The following points were made in the discussion.

It was necessary to increase the concentration of tartaric acid solution to 10 per cent to prevent loss of ergotamine in the ether layer during extraction. The benzhydrol had a low toxicity and did not interfere with the pharmacological effect of ergotamine. Cyclizine was comparatively stable in aqueous solution, and the rate of hydrolysis was slow: tablets would not be affected to any extent.

THE DETERMINATION OF CALCIUM IN HEAVY MAGNESIUM CARBONATE USING GLYOXAL BIS (2-HYDROXYANIL)

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The selective and sensitive colour reaction between calcium ions and glyoxal bis (2-hydroxyanil) has been applied to the determination of 0·1 to 0·5 per cent of calcium in heavy magnesium carbonate. The method, which is simple and rapid, gives results which compare well with those by flame photometry.

SMALL percentages of calcium in materials such as magnesium carbonate or magnesium oxide may be determined by flame photometry more conveniently than by the calcium sulphate precipitation method of the British Pharmacopoeia. Where flame photometers are not available, however, it was thought that the very sensitive colour reaction of calcium ions with glyoxal bis(2-hydroxyanil) might be utilised (Goldstein and Stark-Mayer, 1958).

This reagent reacts with calcium, but not magnesium, to form a bright red complex in strong alkali. Interference from other ions may be suppressed, where necessary, by the addition of carbonate, cyanide or sulphide. Quantitative methods for calcium have been described by Williams and Wilson (1961) and Florence and Morgan (1961); these are based on extraction of the red complex with chloroform and s-octanol respectively. Umland and Meckenstock (1960) have examined the reaction in both a single phase system (water : methanol) and a two phase system (extraction from water with chloroform : hexanol). Kerr (1960) has studied the reaction in a single phase mixture (water : ethanol : butanol).

These spectrophotometric methods were examined and that of Kerr chosen. Solvent extraction methods suffer in that the complex appears to exist in the organic layer as a sol, and once this layer is separated from its aqueous partner the colour becomes very unstable. Kerr's method also reduces the necessary manipulations.

When much magnesium was present, however, the use of a sodium hydroxide/sodium borate buffer, as in Kerr's method, was found unsatisfactory owing to the formation of magnesium hydroxide. It was found preferable to dispense entirely with a buffer solution and add sufficient N sodium hydroxide solution to give optimum sensitivity and stability under the conditions involved.

The assay was also modified to include a blank determination on "calcium free" magnesium carbonate. Initial experiments were made on the effect of alkali concentration and of standing time on the colour development. The position of maximum absorption of the blank solution shifts to shorter wavelengths with time irrespective of the amounts of alkali used. The extinction moreover increases not only with time but

also as the concentration of sodium hydroxide increases. But with 2·8 per cent v/v sodium hydroxide the blank solution has a constant extinction value at 520 mμ up to 2 hr. after mixing. With the assay solution (containing 20 μg. Ca^{++}) the same concentration of alkali gives curves varying with time but coincident between 480 and 560 mμ up to 30 min. after mixing. At 520 mμ curves for both the calcium containing solutions and the "blank" reagent solutions show the greatest difference in extinction. The greatest sensitivity in an assay therefore is expected to be at this wavelength.

It should be observed that at high sodium hydroxide concentrations and on prolonged standing, decomposition of the reagent occurs and it is no longer able to form a characteristic calcium complex.

These initial findings formed the basis of the assay.

TABLE I

RECOVERY EXPERIMENT

0·5 g. "calcium free" magnesium carbonate + *x* mg. of calcium

Wt. calcium taken *x* (mg.)	Per cent calcium	Number of determinations	Average wt. of calcium found (mg.)	Standard deviation
0·20	0·04	8	0·19	0·02
0·40	0·08	7	0·41	0·01
0·80	0·16	10	0·81	0·09
1·00	0·20	7	1·01	0·04
1·20	0·24	2	1·21 1·23	—

Recommended Method

Apparatus. Extinction values were measured in 2·0 cm. cells with a battery operated Unicam S.P. 600 visual range spectrophotometer.

Reagents. Glyoxal bis(2-hydroxyanil) 0·5 per cent w/v in methanol.

Solvent mixture. Equal volumes of 95 per cent ethanol and n-butanol.

Calcium standard solution. Dissolve dry Analar calcium carbonate (1·00 g.) in N hydrochloric acid (25 ml.), boil to expel carbon dioxide, cool and dilute to 1 litre with distilled water. Dilute 10·0 ml. of this concentrated standard solution to 1 litre with distilled water (1 ml. ≡ 4·0 μg. calcium).

Magnesium carbonate "calcium free". Dissolve Analar magnesium sulphate ($7.H_2O$) (24·6 g.) in water (200 ml.) and add dilute sodium hydroxide solution until a precipitate just forms. Heat to 80° and add with stirring a hot solution of sodium carbonate (10·6 g.) in water (200 ml.). Allow the mixture to cool, filter off the precipitate, wash well with water and dry at 110°.

Prepare a standard curve as follows. Dissolve "calcium free" magnesium carbonate (0·5 g.) in 0·5N hydrochloric acid (22·5 ml.), boil the solution for 5 min. to remove carbon dioxide and make up to 250 ml. with water. Place 5·0 ml. of this solution in a series of 8 25-ml. volumetric flasks. Add 0 to 7 ml. calcium standard solution; 7 to 0 ml. of water; 0·70 ml. N sodium hydroxide solution; 0·25 ml. of glyoxal bis(2-hydroxyanil) reagent solution; and 10·0 ml. of ethanol:butanol mixture. Add

water to 25 ml. Mix the solutions well, allow to stand for 15 min. and then centrifuge at 2,000 r.p.m. for 3 min. Measure the extinction of the supernatant liquids at 520 mμ against that of the liquor in an identical experiment but containing no calcium.

Treat heavy magnesium carbonate (0·5 g.) in the same manner as the calcium free magnesium carbonate in the preparation of the standard curve. Day to day reproducibility of the standard curve is poor and therefore standards should be set up at the time of each assay. Should the sample contain an excessive amount of calcium a smaller aliquot must be taken and adjusted to 5·0 ml. with the "calcium free" magnesium carbonate solution.

TABLE II

ANALYSIS OF COMMERCIAL SAMPLES OF HEAVY MAGNESIUM CARBONATE

Sample	By proposed method		Per cent calcium found by	
	Wt. (mg.) of Ca found per 0·5 g.	Per cent calcium	Flame photometry	B.P. method
1	1·12	0·22	0·17	0·14
2	1·35	0·27	0·26	0·22
3	2·40	0·48	0·48	0·40
4	0·86	0·17	0·20	0·14
5	2·36	0·47	0·50	0·44
6	0·96	0·19	0·22	0·16
7	1·05	0·21	0·20	0·18

RESULTS AND DISCUSSION

The standard curve passes through the origin and shows a very slight positive curvature. Sensitivity at the 20 μg. level is 0·023 extinction units per μg. calcium.

Recovery experiments were made on 0·5 g. samples of "calcium free" magnesium carbonate containing known additions of calcium from the concentrated stock solution. A survey of these results is given in Table I. The method has been applied to routine samples of magnesium carbonate and results obtained compared with those by alternative procedures (Table II).

Results obtained agree well with those by a flame photometric procedure but are higher than those by the official method. The present method is rapid and simple, and although the precision is only about $\pm$10 per cent in the worst case, this is considered adequate for the determination of calcium in heavy magnesium carbonate. Where a flame photometer is not available the method should form a useful alternative to the lengthy procedure of the British Pharmacopoeia.

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The paper was presented by THE AUTHOR.

THE ANALYSIS OF POLDINE METHYL METHOSULPHATE BY INFRA-RED SPECTROSCOPY

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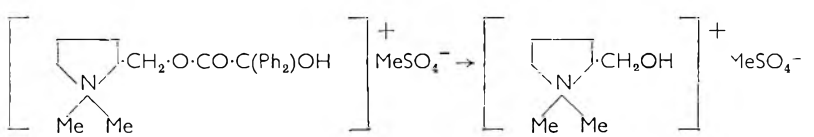
An assay of the drug poldine methyl methosulphate (Nacton, 2-benziloyloxymethyl-1,1-dimethylpyrrolidinium methyl sulphate) is described, based on absorption measurements in the infra-red region of the spectrum. Absorption bands at 5·72, 7·79, 8·13, 9·84, 13·60 and 14·23 μ may be used, the first of which, however, is favoured. The method is no less accurate, and is quicker and more informative than that of Singleton and Wells, with which it is compared. The calculation of drug purity from an absorption band at one wavelength and the use of the ratio of drug purities calculated from the other bands is discussed in relation to standardisation.

The only published method for the assay of poldine methyl methosulphate (Nacton, 2-benziloyloxymethyl-1,1-dimethylpyrrolidinium methyl sulphate) is that of Singleton and Wells (1960). Their method depends on the formation of an ammonium cobaltithiocyanate complex which is extracted by chloroform for absorption measurements at 322 mμ.

The use of the infra-red region of the spectrum, however, might be expected to offer an additional advantage in that several absorption bands, corresponding to different parts of the drug molecule, may be used to obtain further information about the purity of the preparation. It is suggested that the simultaneous use of several bands in this manner merits consideration in drug standardisation. The assay has been devised for the examination of samples taken from bulk supplies of the pure material.

The drug (I) can hydrolyse to give 2-hydroxymethyl-1,1-dimethylpyrrolidinium methyl sulphate (II) and benzilic acid (III), from which it must be distinguished.



I II

+ HO·C(Ph₂)·CO₂H

III

Preliminary investigations showed that presentation of the samples in potassium bromide discs gave variable spectra, and while potassium chloride discs gave better results, they were not adequate for quantitative analysis. The use of a solvent is limited by solubility and absorption considerations, to acetonitrile, in which, however, II is sparingly

soluble (about 0·1 per cent w/w). This limitation is not serious since the drug concentration in acetonitrile is about 2 per cent, and only small amounts of II are likely to be present.

The ester carbonyl absorption band of the drug at 5·72μ is used for assay. Benzilic acid (III), if present, can interfere at this wavelength, but this source of error is readily eliminated by treatment of the assay solutions with sodium bicarbonate before their spectra are obtained.

Experimental and Discussion

Reagents

A.R. sodium bicarbonate (stored over phosphorus pentoxide).

Acetonitrile. B.D.H. analytical reagent grade was used.

Assay Procedure

The drug sample is pulverised in an agate vibration ball mill for 3 min. An accurately weighed portion of the sample of about 60 mg. is weighed into a 10 ml. centrifuge tube to which is added 200 mg. of sodium bicarbonate* and sufficient acetonitrile to make a 2·0 per cent w/w solution of the drug. A control solution without the drug is also prepared. The well-stoppered solutions are allowed to stand, with occasional shaking, for a minimum of 20 min., after which the solutions are centrifuged, transferred to a matched pair of 0·2 mm.† path length cells with sodium chloride windows and scanned in a Grubb Parsons G52A double beam grating spectrometer.

Reference spectra of solutions up to 3 per cent w/w of the pure drug in acetonitrile were thus obtained in the 5–15μ region. Careful standardisation of instrumental conditions was maintained, viz. loop gain, slit width programme and transmission. The spectra are shown in Fig. 1.

The major bands in the spectrum of the drug (I) and their probable assignments are (Bellamy, 1958):

5·72 μ stretching of ester C=O group.
7·99 μ ,, pyrrolidine ring C–N bond.
8·13 μ ,, ester C–O bond.
9·84 μ ,, absorption mainly involving S–O of the methylsulphate ion (by analogy with HSO_4^-, Miller and Wilkins, 1952 c.f. also Chihara 1960).
13·60 μ, 14·23 μ out of plane C–H bond deformation of monosubstituted benzene ring.

It is noticeable that the spectrum of compound III contains absorption bands which interfere with the 5·72, 9·84, 13·60 and 14·23 μ absorption bands of the drug, whilst the spectrum of compound II contains absorption bands which interfere with those of the drug at 7·99, 8·13 and 9·84 μ.

Removal of compound II is difficult, and without the preliminary sodium bicarbonate treatment to remove benzilic acid (III) it appears

* Fig. 2 shows that bicarbonate treatment has no effect on standard solutions of the pure drug.

† Path length was determined by the method of Smith and Miller (1944).

unlikely that a satisfactory assay procedure could be devised. Only the bands at 5·72 μ and possibly 13·60 μ remain for consideration for assay purposes. The former is preferable since it is stronger, sharper and further from a solvent absorption bands.

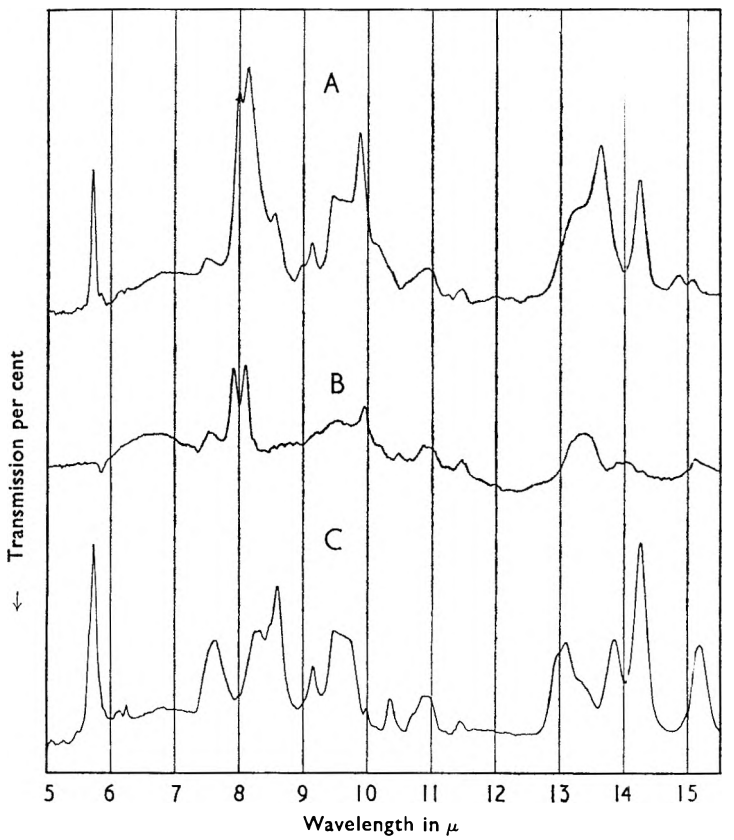


FIG. 1. Infra-red spectra of poldine methyl methosulphate and its hydrolysis products.

A. Poldine methyl methosulphate (2 per cent in acetonitrile.)
B. 2-Hydroxymethyl-1,1-dimethylpyrrolidinium methyl sulphate saturated solution in acetonitrile ($\simeq$ 0·1 per cent w/w).
C. Benzilic acid (2·3 per cent in acetonitrile.)

These reference spectra were used to obtain the absorbances of the various concentrations at the wavelengths of the major bands. Figs. 2 and 3 show plots of unit absorbance (A_s) against concentration. Unit absorbance is calculated thus:

$$A_s = \frac{1}{p}\left(\log_{10}\frac{1}{T} - \log_{10}\frac{1}{t}\right) = \frac{1}{p}\log_{10}\left(\frac{t}{T}\right)$$

where: p = the cell path length (0·212 mm. in the present work).
T = transmittance at the band peak.
t = transmittance at 5·30 μ.

For baseline correction the transmittance at several wavelengths was examined: that at 5·30 μ gave the most reproducible results.

This correction allows for any differences in light scattering, reflection and cell window thickness since both beams of the spectrometer are balanced and the path lengths of the cell are made the same. The very small absorption by the solvent in the regions used greatly facilitates the assay. These factors make it unnecessary to use a geometric construction, as suggested for example by Beaven, Johnson, Willis and

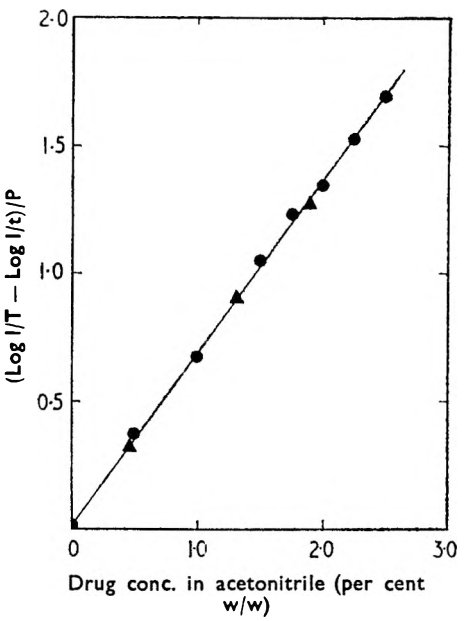


FIG. 2. Calibration curve obtained from absorption measurements at 5·72 μ.

▲, Solution treated with $NaHCO_3$.

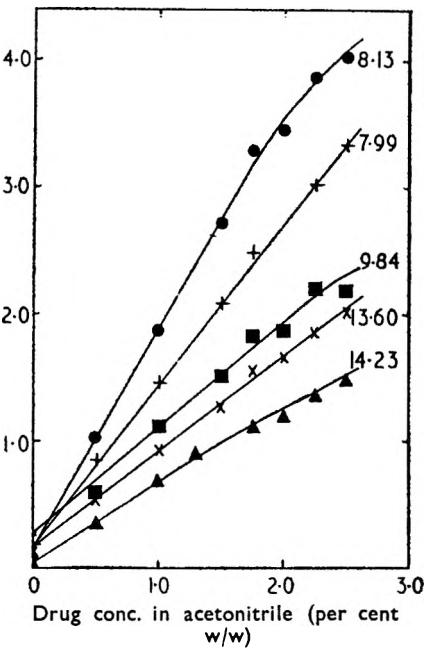


FIG. 3. Calibration curves obtained from absorption measurements at the wavelengths shown (in μ).

Miller (1961), for corrections involving measurements at several wavelengths and the measurement at 5·30 μ is used directly.

The method was tested by assaying known mixtures of the pure drug and compound II or III or both. The mixtures were prepared by mixing the weighed components in an agate mortar using a vibration mill: the 5·72 μ band was used in the absorbance calculation (see above).

The results of these tests (Tables I–II) show an error of $\pm$1·6 per cent with no significant trend.

The method was compared with that of Singleton and Wells (1960); six unknown samples were assayed by two operators, each using one of the methods. The differences between these two (Table III) are about $\pm$1·6 per cent.

TABLE I

ASSAY OF DRUG IN PRESENCE OF COMPOUNDS II AND III

Mixture	Drug per cent	Drug found per cent	Difference
Compound III			
1	100·00	101·3	+1·30
2	97·56	96·3	−1·26
3	94·67	95·6	+0·93
4	91·12	92·5	+1·38
Compound II			
1	100·00	101·3	+1·30
5	97·38	97·7	+0·32
6	94·79	94·3	−0·49
7	89·38	88·7	−0·68

Preliminary work on the assay of the drug in tablets indicates that other substances in the formulation interfere with the 5·72 μ absorption band; calculations based on use of the 7·99 μ band appear to give more consistent results than the 8·13 or 9·84 μ bands.

TABLE II

ASSAY OF DRUG IN PRESENCE OF MIXTURES OF COMPOUNDS II AND III

Mixture	Drug per cent	Compound III per cent*	Drug found per cent	Difference
1	100·0	0	101·3	+1·3
8	87·94	6·47	88·3	+0·36
9	89·09	4·89	89·4	+0·31
10	88·87	7·86	89·8	+0·93
11	87·10	2·69	85·5	−1·6
12	83·60	5·12	82·4	−1·2
13	95·30	2·43	96·4	+1·1
14	93·04	2·69	91·5	−1·54

* The balance is made up of the percentage of II present

Additional Information on Drug Purity

This may be obtained from the other major absorption bands as follows.

An approximately 2 per cent w/w solution of the sample in acetonitrile is made up accurately and the spectrum run in the 5–15 μ region under the same standardised conditions as used for preparing the calibration curves. Sodium bicarbonate is omitted. The percentage purity of the sample is then calculated on each of the six major wavebands using the calibration curves of Figs. 2 and 3.

TABLE III

COMPARISON OF INFRA-RED AND ULTRA-VIOLET ASSAY METHODS

Sample No.	Per cent drug found in assay: Infra-red method operator 1	Ultra-violet method operator 2	Difference
A1	99·3	99·0	+0·3
A2	100·0	100·5	−0·5
A3	100·0	101·2	−1·2
B1	99·0	99·9	−0·9
B2	98·5	99·8	−1·3
B3	99·0	99·9	−0·9

The results obtained from the spectra of the ternary mixtures before treatment with sodium bicarbonate (Table IV) show a considerable variation in the apparent purity.

TABLE IV

DEPENDENCE OF APPARENT PURITY OF DRUG ON ABSORPTION BAND USED FOR CALCULATION

Mixture	Composition of mixture (per cent)			Apparent per cent purity of drug in mixture wavelength of absorption bands used					
	Drug	Compound II	Compound III	5·72 μ	7·99 μ	8·13 μ	9·84 μ	13·60 μ	14·23 μ
8	87·94	6·47	5·59	95·0	96·1	90·4	89·0	82·1	93·5
9	89·09	4·89	6·02	91·7	92·4	90·5	86·0	78·9	81·6
10	88·87	7·86	3·27	95·5	91·0	89·7	87·3	80·8	87·6
11	87·10	2·69	10·21	89·3	96·4	90·0	85·0	80·0	80·0
12	83·60	5·12	11·29	86·3	88·8	88·4	82·0	73·4	78·4
13	95·30	2·43	2·27	96·0	96·4	94·6	88·6	83·9	82·3
14	93·04	2·69	4·27	93·0	95·4	92·5	84·3	80·9	77·5
15	86·88	10·4	2·72	94·5	87·6	86·6	87·1	76·2	89·9

The intensity of the absorption bands at 8·13 μ and 5·72 μ is increased by the presence of compounds II and III respectively. The ratio (r) of the purities calculated from the absorbance at these wavelengths, therefore, should be a function both of the relative amounts of these compounds present and also of the concentration of the drug. For practical purposes in this assay, however, this relationship may be expressed in a two dimensional form (Fig. 4 and Table V).

TABLE V

EFFECT OF COMPOUNDS II AND III ON RATIO OF PURITIES FROM BANDS AT 5·72 μ AND 8·13 μ

Mixture	Drug per cent	Drug per cent from 5·72 μ / Drug per cent from 8·13 μ	Per cent compound III / Per cent compound II
16	86·9	1·09	3·82
11	88·9	1·06	2·40
9	88·0	1·05	1·16
14	95·3	1·01	1·07
1	100·0	1·00	—
10	89·1	1·01	0·83
15	93·0	1·00	0·63
13	83·6	0·98	0·45
12	87·1	0·99	0·26

For the bands at 14·23 μ and 7·99 μ the ratio (r) is apparently very sensitive to the presence of up to 2 per cent of the compounds. If this ratio is plotted against the true purity of the drug (Fig. 4), the curves obtained pass through minima at about 95 per cent purity and rise steeply to the theoretical value of 1·0 for the pure drug.

This kind of ratio can be very useful in problems associated with nearly pure materials.

It can readily be shown that if the assay procedure gives variations of around ±1·5 per cent when applied repetitively to the same sample, then the resulting variation in the ratio is about ±0·04 per cent,

so that the use of such a ratio will tend to offset the variations in procedure and instrumentation invariably occurring from laboratory to laboratory.

In general, it is felt advantageous to assay the purity of a drug using one absorption band, and, to calculate a ratio, using two other carefully selected absorption bands. The choice of these bands depends on the nature of the problem for which the assay is required.

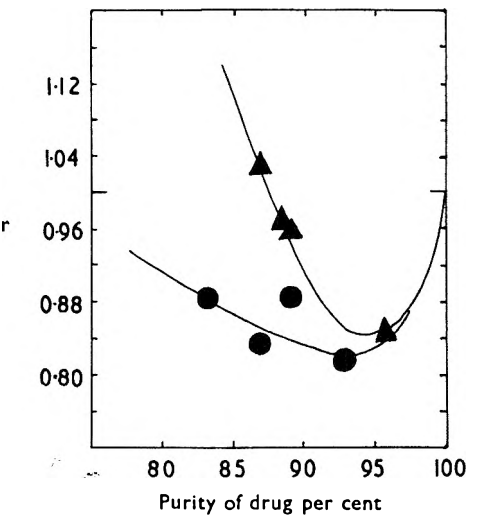


FIG. 4. The effect of compounds II and III on the purity ratio r calculated from absorption measurements at 7·99 and 14·23 μ.

● Compound II predominant. ▲ Compound III predominant.

Acknowledgements. We thank Mr. R. Edser and Miss M. Tingley for assistance in the experimental work and Mr. D. F. Lawson, F.R.P.S., F.I.B.P., F.Z.S., for photographing the spectra and graphs.

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The paper was presented by DR. RAPSON.

THE COLORIMETRIC DETERMINATION OF PHENOLPHTHALEIN

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The conditions governing the intensity and stability of the pink colour given by phenolphthalein in alkaline solution have been examined and a procedure for its colorimetric determination proposed. The results of collaborative trials of this method and of its application to certain pharmaceutical preparations are presented.

DURING revision of the B.P.C. 1954, difficulties with the gravimetric assay of phenolphthalein in Emulsion of Liquid Paraffin and Phenolphthalein made it necessary to investigate an alternative procedure, and a colorimetric method appeared to offer the most appropriate solution to the problem. In the present paper, conditions governing the development of the pink colour of phenolphthalein in alkaline solution have been investigated.

EXPERIMENTAL

Initially, the procedure of Frederick and Koff (1946) was examined, in which a solution of phenolphthalein in ethanol is treated with 0·5N ethanolic potassium hydroxide. Differences in the intensity of the colours produced by different batches of phenolphthalein were excessive however, and agreement between different laboratories with the same sample was poor. Typical figures are shown in Table I.

TABLE I

RESULTS FOR PHENOPHTHALEIN OBTAINED BY THE METHOD OF FREDERICK AND KOFF (1946)

	E (1 per cent, 1 cm.) at max. 560–564 mμ	
Sample	Laboratory A	Laboratory B
1	273·4 267·5	297·6 318·6
2	262·2	311·1
3	252·5 277·0	291·8 314·8

An attempt was then made to apply the method of the Bureau of Industrial Alcohol (1931) in which an aqueous solution of phenolphthalein containing 5 per cent ethanol is made alkaline with 10 per cent ammonia. This gave a considerably greater colour intensity than the method of Frederick and Koff. Values for *E* (1 per cent, 1 cm.) at the maximum at about 550 mμ ranged from 977 to 1002; the colour, however, was not sufficiently stable for routine use. Consideration of these findings, combined with the known instability of the colour given by aqueous sodium

hydroxide, prompted an examination of the effect of pH on the intensity and stability of the colour of alkaline phenolphthalein. During this it was found that even small amounts of ethanol in the final solution markedly reduced the colour intensity, and preliminary evaporation to dryness of all alcoholic solutions was carried out. If, however, this evaporation with ethanol is omitted, maximum colour development is not achieved (but see Discussion).

Nine samples of phenolphthalein from several sources were examined using glycine/sodium hydroxide buffer solutions in the pH range from 10·0 to 12·5; the extinctions obtained with a typical selection of these are given in Table II.

TABLE II

E (1 PER CENT, 1 CM.) OF PHENOLPHTHALEIN AT VARIOUS pH VALUES

	pH										
Sample	10·0	10·45	10·7	10·9	11·1	11·3	11·5	11·7	11·9	12·1	12·5
A	862	1012	1046	1052	1056	1056	1056	1052	1051	1036	964
B	858	1010	1048	1057	1061	1061	1061	1061	1057	1034	948
C	820	990	1053	1053	1055	1055	1055	1051	1047	1018	907

In the pH range where extinction values were greatest, the colour is stable for periods up to 15 min.; after 30 min., the intensity had fallen by about 3 per cent.

The recommended method of colour development is given below. Under these conditions, the curve relating intensity of colour produced to the weight of phenolphthalein up to 0·9 mg. is rectilinear.

Reagents

Buffer solution (pH 11·1). Mix a solution containing 7·5 g. of aminoacetic acid and 5·8 g. of sodium chloride per litre with an equal volume of 0·1N sodium hydroxide. The pH of this solution should be checked electrometrically, and, if necessary, adjusted to 11·1.

Ethanol. 95 per cent Industrial Methylated Spirit.

Procedure

Evaporate a volume of ethanol containing about 0·5 mg. of phenolphthalein to dryness in a small beaker on a boiling water-bath. Dissolve the residue in buffer solution and transfer to a 100 ml. calibrated flask; wash the beaker with successive quantities of buffer solution, adding the washings to the calibrated flask until a volume of 100 ml. is obtained. Measure the extinction of a 1 cm. layer of this solution at the maximum at about 555 mμ. This measurement must be completed within 10 min. of the first addition of buffer solution to the residue of phenolphthalein. For the purposes of calculation, assume the *E* (1 per cent, 1 cm.) of phenolphthalein under these conditions to be 1055.

This method has been applied to pharmaceutical formulations as follows:

Emulsion of Liquid Paraffin and Phenolphthalein B.P.C.

Transfer about 3 g., accurately weighed to a 100 ml. basin and mix to a stiff paste with about 1 g. of Filtercel.* Add ethanol in increments, continuing the stirring to maintain a uniform smooth paste, until the volume of the mixture is about 25 ml. Transfer with the aid of ethanol to a 40 ml. centrifuge tube and centrifuge at 2500 r.p.m. for 10 min. Decant the clear supernatant liquid into a 100 ml. calibrated flask and wash the basin, centrifuge tube and residue by repeating this procedure with 10 ml. portions of ethanol until the extraction is complete. Add the washings to the 100 ml. flask and dilute to volume with ethanol. Apply the general method for colour development to 5 ml. of this solution.

Compound Pills of Phenolphthalein B.P.C.; Compound Tablets of Phenolphthalein B.P.C.; Tablets of Phenolphthalein B.P.

Weigh and powder 20 pills or tablets. Dissolve as completely as possible in 100 ml. of ethanol, a quantity of the powder expected to contain about 10 mg. of phenolphthalein. Allow any insoluble matter to settle and apply the general method for colour development to 5 ml. of the clear supernatant liquid.

Chocolate Preparations

Reduce the sample to coarse granules and chill by immersing in a freezing mixture until brittle. Grind the sample to a fine powder with a chilled pestle and mortar, transfer an accurately weighed quantity of the powder, expected to contain about 20 mg. of phenolphthalein, to a prepared Gooch crucible and extract the fat with three portions, each of 5 ml., of carbon tetrachloride, using slight suction, if necessary, towards the end of the extraction. Extract phenolphthalein from the residue with about 100 ml. of hot ethanol, applied in successive portions, until extraction is complete; transfer the mixed extracts to a 200 ml. calibrated flask, cool and dilute to volume. Apply the general method for colour development to 5 ml. of this solution.

TABLE III

E (1 PER CENT, 1 CM.) VALUES OBTAINED BY THE RECOMMENDED METHOD

Sample	Laboratory		
	A	B	C
British A	1054	1056	1053
,, B	1056	1059	1055
,, C	1062	1060	1062
Italian	1055	1068	1057
German	1058	1063	1057
Origin unknown (at least 8 years old) ..	1055	1053	1055

RESULTS AND DISCUSSION

Samples of phenolphthalein from various sources have been examined in three laboratories by the recommended method. The results on six are given in Table III.

* Johns-Manville Co. Ltd.

From all the figures obtained in the three laboratories, comprising a total of 57 determinations on 39 different samples, the mean value for the E (1 per cent, 1 cm.) of phenolphthalein at the maximum at about 555 mμ is 1055·35 with a standard deviation of 3·84.

Similar extinction values to those quoted above can be obtained by using acetone in place of ethanol in the preliminary evaporation stage; indeed, the same effect can be achieved by heating untreated phenolphthalein in the buffer solution. Dissolving phenolphthalein in the buffer solution without heating, however, gives low and variable extinction values. Moreover, when a solution prepared by any of these methods has faded on standing, the full intensity can be restored by heating in a water bath for 5–10 min. In the recommended methods, ethanol is preferred to acetone because of its more selective solvent properties.

We consider it to be of sufficient importance to repeat our statement that the presence of even a few per cent of ethanol in the final solution is sufficient to reduce the intensity of the pink colour to a marked extent and it is therefore essential that all traces of the solvent be removed at the evaporation stage.

TABLE IV

APPLICATION TO PHARMACEUTICAL PREPARATIONS

	Laboratories			Nominal content
	A	B	C	
Emulsion of Liquid Paraffin and Phenolphthalein B.P.C.				0·36 per cent w/w
Manufacturer I	0·370	0·375	0·360	
II	0·342	0·340	0·341	
Compound Pills of Phenolphthalein B.P.C.				32·4 mg./pill
Manufacturer I	31·5	33·1	32·2	
II	30·6	31·3	31·1	
Compound Tablets of Phenolphthalein B.P.C.				32·5 mg./tablet
Manufacturer II	33·5	34·4	33·5	
II	31·4	31·8	31·0	
Tablets of Phenolphthalein B.P.				129·6 mg./tablet
Manufacturer I	129	130	129	
II	126	128	127	
Chocolate Laxative Type (a)	7·85	8·10	7·97	8·0 per cent
Type (b)	3·87	3·80	3·77	4·0 per cent

The effect of temperature on the colour intensity is negligible over the range of 8° to 28°.

No interference with the determination of phenolphthalein was experienced from other constituents present when the methods described above were applied to pharmaceutical preparations from each of two manufacturers. All were assayed in each laboratory and the results are given in Table IV.

Acknowledgements. We wish to express our thanks to Miss C. J. Lloyd and Miss M. Warrenne for considerable technical assistance thoughout.

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The paper was presented by MR. ALLEN.

THE USE OF TETRAPHENYLBORON FOR THE DETERMINATION AND CHARACTERISATION OF ORGANIC BASES IN PHARMACEUTICAL PREPARATIONS

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A method has been developed for the assay of basic nitrogen compounds by precipitation at pH 3·7 with sodium tetraphenylboron; excess reagent is then determined by back titration with a quaternary ammonium salt. Melting-points of the organic tetraphenylboron salts may be used for the identification of the bases concerned. The method has been applied to the determination of 15 compounds in a variety of pharmaceutical preparations. It compares well in accuracy and speed with existing methods.

Sodium tetraphenylboron, well known as a reagent for potassium, has also been used for the identification and determination of organic bases. Schultz and Mayer (1952) suggested a gravimetric method whilst volumetric procedures involving argentometric determination of organic tetraphenylboron salts have been described by Keller and Weiss (1957) and by Rüdorff and Zannier (1952 and 1954). In our hands these methods proved unsuitable for application to pharmaceutical preparations containing small amounts of bases. The alkalimetric micromethod of Flaschka, Holasek and Amin (1954) is suitable, but involves destruction of the organic tetraphenylborate, which would otherwise be useful for characterisation purposes. Schall (1957) described an indirect volumetric method for the determination of potassium in which precipitation with sodium tetraphenylboron is carried out at pH 12, excess of the reagent then being titrated with a quaternary ammonium salt at the same pH. This principle has been developed to give a method suitable for the determination and characterisation of organic bases in pharmaceutical preparations.

Experimental

Cetylpyridinium chloride (CPC) was chosen as the quaternary ammonium titrant and preliminary work was carried out with 0·01M solutions. Difficulties in preparing these accurately, due to excessive frothing, were overcome by dissolving the CPC in a little ethanol before diluting to volume with water. 0·01M tetraphenylboron (TPB) was prepared using the technique described by Cluley (1955), adjusting the pH to 8·0–9·0 for maximum stability (Cooper, 1957). Of the various indicators in the aminoazo, sulphophthalein and fluorescein groups, bromophenol blue gave the best end-point in acid solution and was sufficiently sharp to permit the use of 0·005M CPC. The optimum pH for this titration is 3·7, the observed "middle tint" of the indicator, although variations between 4·1 and 2·6 can be tolerated. A volume of 0·5 ml. of indicator is necessary for a clear colour change; it must be accurately measured since it introduces a blank of about 0·1 ml. of titrant.

In general, precipitation of organic tetraphenylborates is carried out between pH 2 and 6 and between 20° and 70°. pH 3·7 and 20° were chosen since these conditions applied to the subsequent titrations. Under these conditions semi-colloidal precipitates which are difficult to filter are obtained. Schultz and Goerner (1953) overcame this difficulty by adding aluminium chloride, but this could not be used here because the quaternary ammonium tetraphenylborate formed during the titration tended to coagulate, to absorb the indicator, and to cause a marked deterioration in the end-point. Since hydrophobic sols are less susceptible to coagulation by monovalent than by trivalent ions, sodium chloride was used instead. At a concentration of 1 per cent w/v of the total precipitation volume this allowed ready filtration of the organic tetraphenylborates without affecting the subsequent titration. Under the conditions described below, complete precipitation of all the compounds

TABLE I

APPLICATION TO OFFICIAL SUBSTANCES

Compound	Per cent w/w compound found by		Approximate[1] melting-points of tetraphenyl-boron salts °C
	Proposed method	Alternative method	
Atropine	100·3; 100·3	100·2[1]	160
Atropine sulphate	98·3; 98·2	98·3[1]	160
Homatropine hydrobromide	100·7; 100·8	99·9[1]	160
Atropine methonitrate	100·2; 100·0	99·9[1]; 100·1[2]	*—
Hyoscine hydrobromide	99·2; 99·1	—	104
Lachesine hydrochloride	99·8; 99·6	99·1[1]	164
Physostigmine salicylate	98·2; 98·5	—	109
Pilocarpine nitrate	99·2; 99·2	—	85
Cocaine hydrochloride	99·7; 99·8	99·7[2]	99
Lobeline hydrochloride	99·4; 101·0	—	93
Morphine sulphate	98·8; 98·8	98·6[1]	*—
Codeine phosphate	99·7; 100·0	99·3[1]	*—
Methadone hydrochloride	100·6; 100·8	100·2[1]	83
Pethidine hydrochloride	99·7; 99·7	100·3[1]	155
Procaine hydrochloride	100·9; 101·6	100·5[1]	145

* Decomposed with charring at about 170°–180°.

[1] Official method of the British Pharmacopoeia.
[2] Non-aqueous titration.

listed in Table I takes place within 5 min., with the formation of 1 : 1 complexes. It has been found convenient to add the sodium chloride with the standard solution of TPB. The amount of TPB in excess can be varied between 46 and 120 per cent without affecting the accuracy. The procedure is as follows.

METHOD

Reagents

Bromophenol blue solution of the British Pharmacopoeia, Appendix 2B.

Concentrated buffer solution pH 3·7. Dissolve anhydrous sodium acetate (analytical reagent grade) (10 g.) in distilled water (approximately 300 ml.); add bromophenol blue solution (1 ml.) and sufficient glacial acetic acid (35 to 40 ml.) until the indicator changes from blue to a pure green. Dilute to 500 ml. with distilled water.

Dilute buffer solution pH 3·7. Dilute concentrated buffer solution with an equal volume of distilled water.

0·005M CPC. Dissolve cetylpyridinium chloride (1·80 g.) in 95 per cent ethanol (10 ml.) and dilute to 1 litre with distilled water. Store in an amber bottle.

TABLE II

APPLICATION TO AQUEOUS EYE-DROP PREPARATIONS

Eye-drops	Volume of sample (ml.)	Dilution to* (ml.)	Per cent w/v compound	
			Present	Found by proposed method
Atropine sulphate B.P.C.	4	20	0·98	1·00; 0·98 0·98; 0·98 0·98
Homatropine B.P.C.	3	20	1·99	2·01; 2·00
Cocaine B.P.C.	3	20	1·99	1·99; 1·98
Pilocarpine B.P.C.	4	20	1·00	0·99; 0·99
Atropine methonitrate B.N.F. ..	4	20	1·00	1·00; 1·00
Physostigmine B.P.C.	5	10†	0·49	0·50; 0·51 0·50
Lachesine B.P.C.	4	20	0·98	0·98; 0·98
Hyoscine B.P.C.	5	10†	0·246	0·241; 0·247

* With dilute buffer solution except those marked †, where concentrated buffer solution is used.

0·01M TPB. Dissolve sodium tetraphenylboron (3·42 g.) in distilled water (50 ml.), add moist aluminium hydroxide gel (0·5 g.) and shake for 20 min. Dilute to 300 ml. with distilled water; dissolve sodium chloride (16·6 g.) in this solution and stand for 30 min. Filter clear,* under suction, through two thicknesses of No. 42 Whatman filter paper. After washing the filter, dilute the filtrate to 1 litre with distilled water and adjust the pH to 8·0 to 9·0 with 0·1N sodium hydroxide using narrow range Universal pH papers. Store in an amber bottle.

TABLE III

APPLICATION TO INJECTION SOLUTIONS

Injection solutions	Volume of sample (ml.)	dilution to* (ml.)	Compound	
			Present	Found by proposed method
Lobeline hydrochloride B.P.C. 3 mg./ml.	5	10†	2·96 mg./ml.	2·99 mg./ml.
Methadone B.P. 1·00 per cent w/v ..	4	20	1·01 per cent w/v	1·01 per cent w/v
Morphine sulphate B.P. 32·4 mg./ml. ..	2	20	32·6 mg./ml.	32·7 mg./ml.
21·6 mg./ml. ..	3	20	21·5 mg./ml.	21·4 mg./ml.
Pethidine B.P. 5·0 per cent. w/v .. .	1	20	5·03 per cent w/v	5·02 per cent w/v
Procaine and Adrenaline B.P.	2	20	2·03 per cent w/v (Procaine hydrochloride)	2·06 per cent w/v

* With dilute buffer solution except those marked †, where concentrated buffer solution is used.

Both the CPC and TPB solutions deteriorate slightly on standing; they should be standardised at frequent intervals.

0·01M *Potassium chloride solution.* Dissolve analytical reagent grade potassium chloride (0·1491 g.), previously dried at 150° for 1 hr., in dilute buffer solution (200 ml.).

Sample Preparation

Pure compounds. Prepare an approximately 0·01M solution in dilute buffer solution.

Aqueous eye-drops and injection solutions. Dilute with buffer solution as directed in Tables II and III; use 10 ml. for the assay.

* Re-filter the first 20-30 ml. of filtrate if cloudy.

Tablets (containing lactose basis). Dissolve the powdered tablets, with gentle warming, and dilute to the volume given in Table IV; centrifuge if necessary; use 10 ml. for the assay.

Suppositories, eye-ointments and oily eye-drops. Dissolve the preparation, with warming, in the specified organic solvent and extract as directed in Table V. Filter each extract in turn through a small plug of cotton wool. Gently warm the combined extracts, to remove traces of organic solvent, and then dilute to volume with concentrated buffer solution; use 10 ml. for the assay.

TABLE IV

APPLICATION TO SIMPLE UNCOATED TABLETS

Tablets	Sample preparation		Compound found by	
	No. of tablets	Dilution to* (ml.)	Proposed method	Alternative method
Hypodermic tablets—				
Atropine sulphate 1 mg.	40	20	1·06 mg./tab.	1·03 mg./tab.[1]
Pilocarpine nitrate 0·65 mg.	6	20	0·63 mg./tab.	0·62 mg./tab.[1]
Morphine sulphate 16·2 mg.	5	25	15·7 mg./tab.	16·1 mg./tab.[2]
Ophthalmic tablets—				
Cocaine hydrochloride 5 mg.	14	20	5·05 mg./tab.	4·98 mg./tab.[2]
Simple tablets—				
Atropine sulphate B.P. 0·65 mg.	40	25	1. 0·64 mg./tab. 2. 0·67 mg./tab.	0·61 mg./tab.[3] 0·64 mg./tab.
Pethidine B.P. 25 mg.	2	25	23·7; 24·0 mg./tab.	23·9 mg./tab.[3]
Codeine phosphate B.P. 32·4 mg.	2	25	33·2 mg./tab.	32·8 mg./tab.[3]
16·2 mg.	4	25	17·0 mg./tab.	16·9 mg./tab.
Hyoscine B.P. 0·65 mg.	40	25	0·61 mg./tab.	0·61 mg./tab.[3]

* With dilute buffer solution.

[1] The method described for Atropine Sulphate Tablets B.P. 1958.
[2] U.V. Spectroscopy.
[3] Official method of the British Pharmacopoeia.

Procedure

Transfer the prepared solution (10 ml.) to a clean, dry beaker, add 0·01M sodium tetraphenylboron (15 ml.) accurately measured, while swirling the contents of the beaker, and allow to stand for 5 min. Filter through a dry sintered-glass funnel (porosity 4) under gentle suction into a dry flask. (Reserve the residue for identification purposes.) Transfer exactly 20 ml. of the filtrate to a 150 ml. flask, add bromophenol blue solution (0·5 ml.), accurately measured, and titrate with 0·005M cetylpyridinium chloride to a blue end-point ('a' ml.). To a further 15 ml. of sodium tetraphenylboron, add concentrated buffer solution (4 ml.) followed by bromophenol blue solution (0·5 ml.), accurately measured, and titrate as above to the same end-point ('b' ml.).

The difference in titres $(b - \frac{5a}{4})$ ml. is equivalent to the volume of 0·005M sodium tetraphenylboron precipitated by the organic base.

At the same time, determine the molarity of the cetylpyridinium chloride solution by pipetting 0·01M potassium chloride (10 ml.) into a clean, dry beaker and continuing as above from the words "add 0·01M sodium tetraphenylboron (15 ml.) . . . titrate with 0·005M CPC to a blue end-point" ('c' ml.).

TABLE V

APPLICATION TO MISCELLANEOUS PREPARATIONS

Preparation	Sample preparation				Compound found by	
	Quantity of preparation	Organic solvent	Extraction	Dilution to* (ml.)	Proposed method	Alternative method
Suppositories of morphine B.P.C. 16·2 mg.	4 suppositories	10 ml. light petroleum (b.p. 40°–60°)	1 × 10 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	25	16·1; 16·1 mg. suppository	15·0 mg. suppository[1]
Suppositories of morphine B.P.C. 64·8 mg.	1 suppository	10 ml. light petroleum (b.p. 40°–60°)	1 × 10 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	25	61·6; 60·3 mg. suppository	68·0 mg. suppository
Oily eye-drops of atropine 1 per cent w/v ..	5 g.	5 ml. solvent ether	1 × 5 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	20	1·00; 1·00 per cent w/v	1·00 per cent w/v[2]
Oily eye-drops of physostigmine 1 per cent w/v	5 g.	5 ml. solvent ether	1 × 5 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	20	0·99; 1·00 per cent w/v	1·00 per cent w/v[2]
Atropine eye ointment 1 per cent w/w ..	8 g.	5 ml. solvent ether	1 × 15 ml. 2N acetic acid 2 × 10 ml. and 1 × 5 ml. concentrated buffer	50	1. 0·97; 0·98 per cent w/w	0·98 per cent w/w[3]
					2. 0·96; 0·96 per cent w/w	0·98 per cent w/w

* With concentrated buffer solution.

[1] Nitroso morphine method after extraction.
[2] Laboratory prepared.
[3] Official method of the British Pharmacopoeia.

The molarity of the CPC is then given by the relationship $M = \frac{10\,M'}{(b - \frac{5c}{4})}$ where M′ is the molarity of the potassium chloride solution.

The residue obtained in the above assay may be used for the determination of melting-point as follows.

Wash the residue with distilled water (5 portions of 20 ml.) and dry over phosphorus pentoxide at a pressure not exceeding 5 mm. Determine the melting-point by Method I, Appendix IVA of the British Pharmacopoeia.

Results and Discussion

The recommended method has been applied to the determination of 15 basic nitrogen compounds. The results together with those obtained by alternative procedures are given in Table I. This also lists melting-points of the tetraphenylborates which are of value for identification purposes. Since some organic tetraphenylboron salts are thermally unstable (Wendlandt and Dunham, 1958) the conditions of the British Pharmacopoeia for the determination of melting points must be closely followed. In a number of instances it is possible to carry out supplementary chemical identification tests on the residue; for example, the Vitali test can be applied directly to the atropine derivative.

Applications to aqueous eye-drop and injection solutions are given in Tables II and III. In the study of interfering substances it has been established that esters of *p*-hydroxybenzoic acid, phenol, chlorbutol, chlorocresol and chloroxylenol in concentrations at which these materials are used as fungistats, bactericides and bacteriostats do not interfere. Phenylmercuric nitrate (0·002 per cent w/v) causes a small positive error. The extent of this error will depend on the ratio of phenylmercuric nitrate to active ingredient present and will usually lie between 0·2 and 1 per cent.

Solutions prepared by treating up to 1 g. of lactose, liquid glucose, mannitol, stearic acid, starch, calcium stearate, talc and sucrose by the method recommended for tablets produce no interference. Gelatin and polyvinylpyrrolidone precipitate with TPB and thus invalidate direct application of the method. Table V shows the application of the method to a number of preparations containing oils and fats. Acid extracts, prepared as directed in the method, from Theobroma Oil B.P.C., Basis for Eye Ointment, B.P. and Castor Oil B.P. contained no interfering materials.

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The paper was presented by Mr. King.

WATER FOR INJECTION BY ION-EXCHANGE

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The purification of water by means of ion-exchange resins has been recognised as satisfactory for general purposes by a number of Pharmacopoeias (B.P., 1958; U.S.P., 1960; see also Saunders, 1954). The disadvantage of the method is that the freshly purified water may be contaminated by bacteria and consequently cannot be used for preparing solutions for injection (Whittet, 1961).

The object of this work has been to study the conditions required to give a reliable source of apyrogenic deionised water which can be used for all purposes where bacteriologically clean water is required. The advantage of the ion-exchange purification is that large quantities can be prepared rapidly as required, the small plant used in these experiments could supply 40 litres/hr.

Bacterial Counts on Water Samples

These were performed by spreading five 0·5 ml. water samples over the surface of over-dried tryptone soya agar plates. The more heavily contaminated samples were diluted 100 times with sterile distilled water before counting. For the less heavily contaminated waters, five samples each of 20 ml. were inoculated into 20 ml. of double strength liquid culture medium and the results read as growth or no growth. This latter procedure is a test several times more stringent than the British Pharmacopoeia test for sterility.

All samples with appreciable contamination, showed a mixed flora with Gram-negative rods and micrococci predominating; many contained Gram-positive sporing rods. The sample of London tap water used as influent in the later experiments came from a roof storage tank and was contaminated with a Gram-positive sporing organism which provided an efficient test for the subsequent experiments since it appeared to be resistant to ultra-violet and chemical sterilisation and readily grew in purified water on storage at room temperature for several days.

The results showed that stored distilled water often gave counts of above 10^4 organisms/ml.

Ion-Exchange Purification of Water

To prepare purified water with negligible bacterial contamination by ion-exchange, the de-ionising equipment must be completely freed from micro-organisms. It is then preferable to have a sterile feed water supply otherwise colonies begin to grow in the resin column and eventually appear in the effluent.

The de-ioniser used in this work was a modified Elgastat B112 UV in which the influent water passed first through the outer jacket of a double

jacketted ultra-violet lamp, then to a polythene bottle containing 8 litres of mixed ion-exchange resins and on through the inner jacket of the lamp and a conductivity cell to the effluent pipe, the tip of this pipe was immersed in a 1 per cent formaldehyde solution when not in use. The thickness of each of the water layers surrounding the lamp was about 1 mm.

Sterilisation

At reasonable flow rates the ultra-violet lamp alone could not be relied upon to give a sterile effluent. Initial chemical sterilisation of the whole apparatus was achieved by filling it with 1 per cent formaldehyde solution and leaving overnight. Subsequent washing with about 60 litres of water was necessary to remove the formaldehyde.

With London tap water feed, purified water with a count of less than one organism per 20 ml. and a negligible pyrogen reaction was obtained. When the water was stored for a week, however, an appreciable growth of micro-organisms occurred due probably to the presence of spores in the feed water which had survived passage through the ultra-violet lamp.

To overcome this difficulty, the feed water was treated with an ionic sterilising agent—acidified sodium hypochlorite—which is removed by the resins. The influent tap water was run into a 20 litre polythene aspirator and sodium hypochlorite solution acidified with hydrochloric acid was added to give a concentration of about 80 p.p.m. of free chlorine. The chlorinated water stood overnight before use.

The resulting purified water collected one week after the formaldehyde sterilisation had a count of less than one organism per 100 ml. and a negligible pyrogen reaction. One month after the formaldehyde sterilisation the pyrogen reaction of the purified water was still well within the Pharmacopoeia limits (average temperature rise of 0·23°) but it gave a small count of about five organisms/ml. This effect must have been due to some highly resistant spores in the feed water and could be avoided by using a higher concentration of chlorine.

Conclusions

The total capacity of the ion-exchange cartridge for London tap water is about 250 litres; of this about 40 per cent had to be invested in washing the apparatus free from formaldehyde and in removing the sodium hypochlorite.

The technique described could be used with an ion-exchange plant in which regeneration of the resins is carried out. After regeneration the whole plant should be sterilised with formaldehyde and then washed through with chlorinated water, subsequently all water admitted to the apparatus should be chlorinated and ultra-violet irradiation of both influent and effluent water is recommended.

The following arrangements should give a reliable source of apyrogenic, purified water with a negligible bacterial count.

1. A column of mixed ion-exchange resins is supplied with chlorinated feed water from a tank of volume equal to the capacity of the resins for

the raw water used. Both influent and effluent water are irradiated by ultra-violet light.

2. The whole ion-exchange apparatus is sterilised either by filling it with 1 per cent formaldehyde solution or by drawing moist formaldehyde vapour through it, the latter method reduces the volume of wash water required.

3. When not in use the outlet pipe of the apparatus is kept immersed in formaldehyde solution to prevent entry of micro-organisms.

The apparatus should be sterilised at weekly intervals and the resin bed volume should be such that the volume of purified water required per week is three-fifths of the capacity of the de-ioniser, for the feed water used.

The authors consider that a modification might now be made to the monograph in the British Pharmacopoeia on Water for Injection, so as to permit its preparation by ion-exchange under suitably controlled conditions.

Acknowledgements. The authors thank Elga Products Ltd. of Lane End, Bucks, for the loan of the ion-exchange equipment, they also thank Mr. W. Lorch for his suggestions and Mr. H. Fearn of the Pharmacology department, School of Pharmacy, for carrying out the pyrogen tests.

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Discussion

The paper was presented by Professor Cook. The following points were made in the discussion.

The authors were not satisfied that the formaldehyde was killing all the spores in the column. Chlorination was the most efficient method of sterilisation. Other processes with chemicals were unsuitable. Irradiation with ultra-violet light was only an aseptic precaution; there was a risk of ozonolysis or peroxide formation with ultra-violet light, and this might be significant in the preparation of injections of phenothiazine compounds having a free sulphur atom because the sulphoxides formed would inactivate the compounds. Water was more likely to be contaminated by colonies or organisms rather than by a single organism, and for that reason a filtration method of sterilisation would probably be more reliable than ultra-violet light. Multi-layer filters with built-in heaters were available and these could be sterilised *in situ*. On one occasion, gamma-radiation had been used successfully to sterilise an ion-exchange column. The conditions in a mixed-bed resin, which had been reported to give pyrogen-free water, could differ from those in a cartridge type of apparatus such as had been used. The frequency of flow through the column was of

importance; if the flow was stopped for 24 hr. pyrogenic water was produced. The water from the apparatus as well as being pyrogen-free was also satisfactory both physically and chemically. Tests for pyrogenicity were made on the feed water, the first effluent, the last effluent, and the first effluent after storage.

SOME PHYSICAL PROPERTIES OF INTERFACIAL FILMS OF POTASSIUM ARABATE

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The rheological properties of the film formed between solutions of potassium arabate and Light Liquid Paraffin B.P. have been investigated. It is found from preliminary experiments that such a film has plasto-elastic properties and that it becomes more rigid with the passage of time.

It was shown by Plateau that the surface of a liquid appears to offer a greater resistance to the motion of a body than does the bulk liquid. Quantitative measurements were attempted by later workers using oscillating disc pendulums; Stables and Wilson studied saponin solutions in 1883 and showed an enormous increase in resistance to oscillation when the pendulum was in the surface rather than above or below it. Wilson and Ries (1923) used a similar method to investigate the physical properties of certain solutions, concluding that a film existed at the surface having the nature of a plastic solid. In 1955, Criddle and Meader using a biconical oscillating pendulum studied the viscosity and elasticity of films, and the effect of ageing on these properties. Before this Tachibana and Inochudi (1953) had made a different approach to the problem. Using a very light wire ring as pendulum they observed its motion through a comparatively small angle under varying degrees of torque applied by a fine torsion suspension, and the results were interpreted in rheological terms rather than as a viscosity. This method has the advantage of causing less disturbance to any structure that may exist at the surface than the oscillation method, and of being able to detect rapid changes in these properties.

Experimental

Two types of system were investigated using a torsion pendulum, that at the aqueous potassium arabate/air interface and that at the aqueous potassium arabate/light liquid paraffin interface. The brass bob was biconical in shape, of 20° included angle and 5·621 cm. diameter. Before being attached to the torsion wire the bob was thoroughly cleaned, immersed for 5–10 sec. in a dilute nitric acid solution until the surface was etched, and then well washed to ensure it was readily wetted by water. It was suspended by a copper beryllium wire 0·015 cm. diameter, 58 cm. long which had been heat treated for 3 hr. at 320° whilst under tension to straighten it and develop its elastic properties. The torsion constant was 10·77 dynes/cm., and the moment of inertia of the rotating system was 47·46 g./cm.^{2}. At its upper end the wire was secured in a collet which could be adjusted vertically by means of a micrometer screw, and rotated through a known angle.

Rotary displacements of the bob were measured by an optical lever of about 2·7 metres. One millimeter deflection on this scale represents a rotation of about 0·01 degree. Precautions were taken to eliminate the effect of backlash on the rotating and measuring parts. Solutions were made in freshly distilled water and placed in scrupulously clean optical cells 6 × 10 × 10 cm.

The apparatus was set up so that the equator of the pendulum bob was brought to the interface by raising or lowering the cell platform using a slow speed electric driving system, care being taken to ensure that the lower surface of the bob was completely wetted, and the upper surface untouched by the gum solution. At this stage the optical lever was adjusted to the zero of the scale. After the lapse of a suitable time, by rotating the torsion head between adjustable stops, a predetermined twist (either 20° or 45°) was put in the wire and the movement of the optical

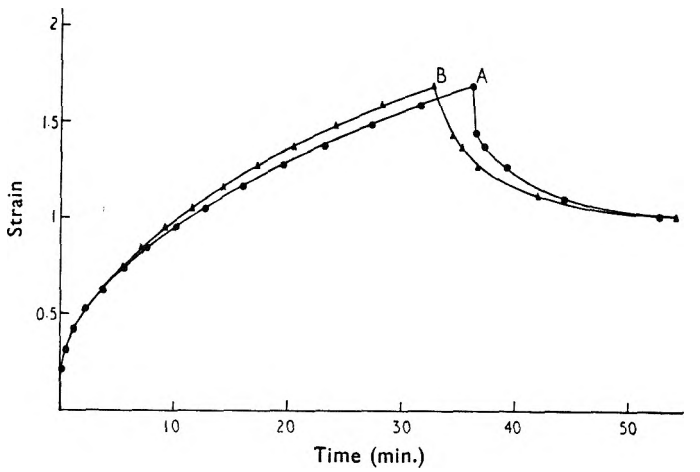

FIG. 1. Strain-time curve for film at interface of 15 per cent potassium arabate solution and A, air; B, light liquid paraffin. Initial torsion 20°.

lever recorded at suitable time intervals. When the bob had rotated about 5° the head was returned to its zero position or to such a position that the wire was free from strain, and subsequent movement recorded. The movement of the pendulum was limited to about 5° of arc to minimise the chance of damage to the film. Only on one occasion, when the degree of wetting of the pendulum was not satisfactory was there any evidence of slip between film and pendulum.

This type of experiment was made on solution/air interfaces about 1 hr. old and on solution/oil interfaces of ages varying between 1 and 66 hr.

Materials

The aqueous phase was a 15 per cent w/v solution of potassium arabate prepared by the method described by White (1960).

The oil phase was Light Liquid Paraffin B.P.

Results

When the pendulum was totally immersed in either the solution or the oil it oscillated freely when displaced from its resting position, but when at the interface it moved only in one direction.

In all cases the results were qualitatively similar, the magnitude of the effect depending only on the age and not on the nature of the interface as shown in Figs. 1 and 2.

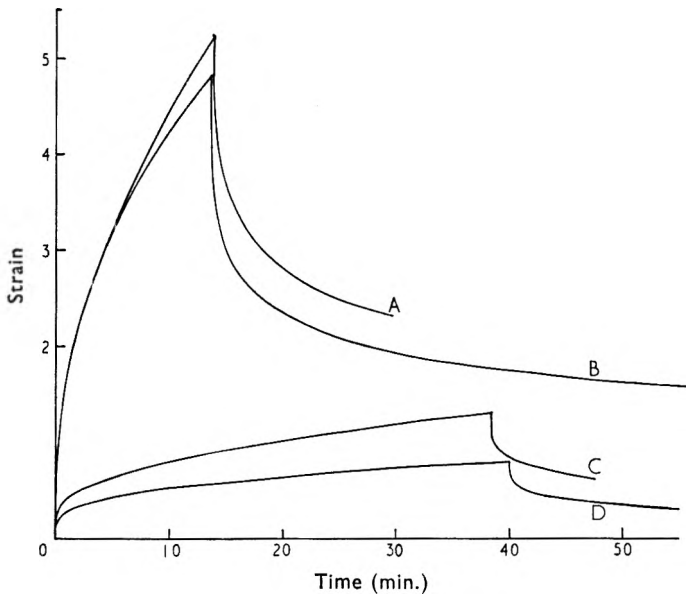

FIG. 2. Strain-time curve for film at the interface of a 15 per cent potassium arabate solution/light liquid paraffin. Initial torque 45°.
Film age: A, 1 hr. B, 1¼ hr. C, 22 hr. D, 66 hr.

Discussion

To produce a stable emulsion it is necessary for the globules of the disperse phase to be prevented from coalescing, this is usually achieved either by causing the droplets to be electrically charged, and so mutually repulsive, or by enclosing them in an integument having the necessary physical properties of rigidity, elasticity and cohesive strength. Acacia appears to function largely by this latter method, it is therefore of interest to determine the rheological properties of the interfacial film.

Measurements of interfacial viscosity may be misleading since the film may not be in the liquid state, but be a semi-solid or solid body. This consideration rules out all methods using either flow or a body oscillating through an appreciable arc since excessive vibration would destroy any film structure. If the amplitude of vibration be kept so small that this danger is minimised, then it becomes difficult to maintain the vibration and to measure its rate of damping. However, under such conditions it becomes possible to measure the angle of displacement of the bob of

a torsion pendulum and the torque necessary to produce it. From the behaviour of the system whilst under stress and after removal of the stress it is a simple matter to describe qualitatively its rheological properties, but whether or no a quantitative interpretation can be made will depend on the events that take place. In particular plastic flow may cause difficulty.

The form of the strain versus time graph (Figs. 1 and 2) is that typical of visco-elastic and plasto-elastic materials but it does not allow distinction to be made between them. In every case there is a rapid displacement of the bob of the pendulum which may represent an instantaneous elastic response damped by the large moment of inertia of the system, and then a rate of deflection decreasing more rapidly than the torque. Removal of the stress allows a rapid recovery and then a decreasing rate of relaxation

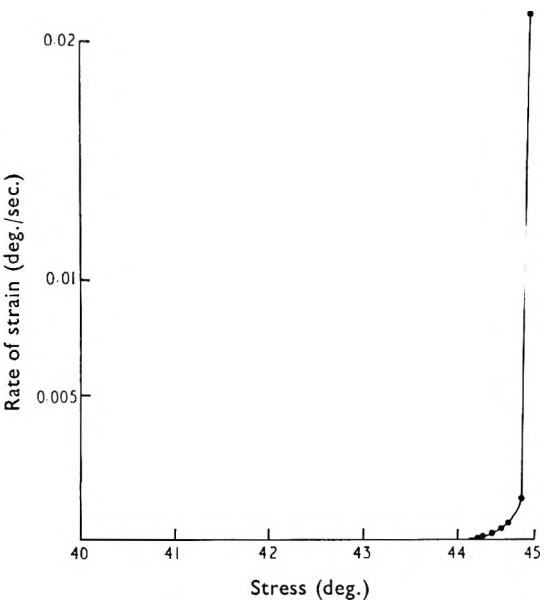

FIG. 3. Rate of shear *vs.* stress for film at interface of 15 per cent potassium arabate/light liquid paraffin.

but in no case did the pendulum return to its original position, clear indication that flow had taken place. Distinction between viscous or plastic flow in the systems can be made by reference to the rate of strain versus stress graph Fig. 3 in which there is an intercept at a substantial value on the stress axis, evidence of the existence of a yield value, hence in the film, of plastic flow. The behaviour of the film under, and free from applied stress, may be depicted in formal fashion with a spring dashpot model incorporating five elements (Fig. 4). This is arranged in three parts and shows the rapid elastic response, the slow recoverable response, and the yield value. Under very low stresses this last element will not function and at higher stresses represents the irrecoverable

element of flow. The whole model simulates the behaviour of a plasto-elastic body. The effect of the lapse of time can be shown by the calculation of an Apparent Shear Modulus by the use of

$$n = \frac{K}{4\pi h} \times \left(\frac{1}{R_1^2} - \frac{1}{R_2^2} \right) \times \frac{\delta - \theta}{\theta},$$

(where K = Wire constant 10·7698 dynes/cm., h = Thickness of film = 1500 Å; R_1 = Radius of pendulum bob; R_2 = Radius of dish; δ = Applied torque; θ = Measured deflection) assuming that no plastic flow has taken place. The effect of such an assumption is to reduce slightly the magnitude of δ and more seriously increase that of θ, resulting in a low value for n. The magnitude of the discrepancy decreases with the decrease of θ, i.e., with the age of the film.

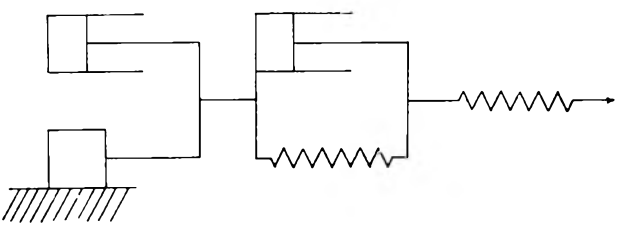

FIG. 4. Spring and dashpot model to represent the behaviour of the potassium arabate film at the oil/water interface.

The only factor in this equation that was not evaluated for this particular case was h the thickness of the film, and for this, White's determination of 0·15 μ for films in the presence of a 15 per cent w/v solution of potassium arabate was used.

TABLE I

APPARENT SHEAR MODULUS OF INTERFACIAL FILMS OF 15 PER CENT W/V POTASSIUM ARABATE/LIGHT LIQUID PARAFFIN

Age of film (hr.)	n
0·5	0·34 × 10^6 dynes/cm.^{2}
1·0	0·32
1·75	0·36
2	0·44
22	1·59
66	2·59

In the case of the film 66 hr. old, since the film reached equilibrium in both parts of the experiment n can be calculated making due allowance for the shift of zero due to the plastic flow, when the value is found to be 4·25 × 10^5 dynes/cm.^{2} and the yield value 1·11 × 10^4 dynes/cm.^{2}. These figures indicate the film to be considerably more rigid than a 10 per cent gelatine gel for which values for n of about 3 × 10^5 dynes/cm.^{2} are quoted by Alexander and Johnson (1950).

The evidence now available shows that solutions of arabates develop an interfacial film as a third phase between the aqueous phase and the

adjacent one be it air or liquid. These films all have well marked time dependent plasto-elastic properties which means that they are able to undergo considerable elastic distortion and when the elastic limit is exceeded the film flows plastically and does not break. In addition, any portion of the film which suffers such thinning will gradually regain its original condition. Such physical properties allied to the substantial thickness of film make arabates almost ideal emulsifying agents, their only shortcomings being their liability to microbial attack.

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The paper was presented by THE AUTHOR.

A NOTE ON THE STABILITY OF SOLUTIONS OF ISOPRENALINE

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ISOPRENALINE Spray Solution (B.P.C.) contains sodium metabisulphite (0·1 per cent) and must be recently prepared or stored protected from light in small well-filled containers. Since metallic ions catalyse the decomposition of isoprenaline (B.P.C. 1958) and since disodium edetate is a better preservative than sodium metabisulphite for solutions of the related phenylephrine (West and Whittet, 1960) comparison of the stabilising power of these two agents has been extended to the Spray Solution of Isoprenaline. Four solutions were prepared: (1) Isoprenaline Spray, B.P.C., (2) Isoprenaline Spray, B.P.C. omitting sodium metabisulphite, (3) Isoprenaline Spray, B.P.C. omitting metabisulphite but containing disodium edetate (0·1 per cent) and (4) Isoprenaline Spray, B.P.C. containing added disodium edetate. Ampoules (5 ml.) of each of these were sealed under air and stored at 15–20° for 16 months. They were then assayed for biological activity using the blood pressure of the anaesthetised cat (depressor action) and the isolated rabbit ileum (inhibitory action). Only solution 3 differed significantly in activity from the standard solution 1: the loss in activity, however, was slight. Both solutions not containing metabisulphite turned light brown in colour.

Although intravenous infusion of isoprenaline has been recommended (Segal, 1960; Hellerstein, 1960) no information is available as to how the solutions are sterilised and the effect of various methods on the stability of isoprenaline has been examined. A solution of isoprenaline sulphate (1 in 10,000) in water for injection containing sodium metabisulphite (1 in 1,000) was prepared. Part of this was sterilised by filtration through a bacteria-proof sintered glass funnel and sealed in sterile 2 ml. ampoules, half of these under air and half under nitrogen. The remaining solution was placed in 2 ml. ampoules, half of which were sealed under air and half under nitrogen; ampoules from both of these two groups were autoclaved at 115° for 30 min., the rest were steamed at 100° for 30 min. Samples from each of the 6 batches were assayed on the isolated guinea-pig heart (cardiac stimulant action) and on the rabbit ileum (inhibitory action). There was no loss of biological activity in any of the solutions. Even after storage at 15–20° for 16 months, there was no significant loss of activity and all solutions remained colourless.

Isoprenaline solutions of this composition showed no incompatibility with chlorocresol 0·2 per cent or phenylmercuric nitrate 0·002 per cent.

Thus, in the Spray Solution of Isoprenaline, disodium edetate does not prevent deterioration; it is not recommended as a preservative for this

solution. For isoprenaline solutions for injection, any one of the B.P. sterilisation methods is satisfactory when sodium metabisulphite is present as an antoxidant.

Acknowledgements. We wish to express our thanks to Mr. L. K. Njikam for carrying out some of the biological assays in the preliminary experiments.

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The paper was presented by DR. WHITTET.

THE ASSAY OF PROTAMINE SULPHATE FOR ITS CAPACITY TO NEUTRALISE HEPARIN

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PROTAMINE sulphate is used clinically to neutralise the anticoagulant effect of heparin, and the B.P.C. includes an *in vitro* method for the assay of Injection of Protamine Sulphate: this is based on the assumption that 1 mg. of protamine sulphate will neutralise 86 units of heparin. Excess heparin, in the mixture of heparin and protamine sulphate used, is determined biochemically, but any physical property of heparin will serve equally well.

Excess heparin or protamine sulphate in mixtures of the two may be demonstrated by the extent to which spots of the mixtures spread when applied to Whatman No. 1 filter-paper as shown by staining with bromocresol green. When protamine is in excess the stained spot is compact and well within the zone wetted during application; when heparin is in excess the staining extends to the limit of the wetted area.

TABLE I

REACTIONS NOTED WITH 1 MG. OF PROTAMINE SULPHATE BATCH 269 WITH VARYING QUANTITIES OF HEPARIN BATCH 3338

Units of heparin	64, 66, or 68	70, 72, or 74
Nature of the spot	Compact	Diffuse
Protamine reaction in supernatant fluid ..	Positive	Negative

The sensitivity and specificity of this change from a compact to a diffuse spot have been examined (Table I). To tubes containing 1 ml. of 0·1 per cent aqueous solution of protamine sulphate, quantities of heparin in aqueous solution were added in increments of 2 units. Drops of these mixtures (0·01 ml.) were applied to Whatman No. 1 paper, the area of wetting being outlined in pencil. The spots were allowed to dry, then stained by immersion in 0·02 per cent bromocresol green for 5 min. followed by washing in 2·0 per cent w/v acetic acid. The specificity of the change was checked by applying the method used by Godal (1960). The tubes containing protamine and heparin were centrifuged at about 16,000 r.p.m. (20 min.) and the supernatant fluids spotted as above. This procedure will detect protamine sulphate in aqueous solution at a level of 5 μg./ml.

A comparison of the amounts of heparin neutralised by 1 mg. of protamine sulphate as determined by this method, and by the method of the B.P.C., is shown in Table II. The values obtained by the two methods do not agree; they also depend on the sample of heparin used. This

illustrates a weakness of the B.P.C. test, which does not call for the use of a specific heparin standard. Although the absolute neutralisation values differ, if one batch of protamine is used as a standard (arbitrarily 100 per cent) and the potency values of the others expressed in terms of this, then the values are roughly equal irrespective of the method or the heparin used. The figures in brackets (Table II) are derived thus.

TABLE II

UNITS OF HEPARIN NEUTRALISED BY 1 MG. PROTAMINE SULPHATE

Protamine Batch	Heparin batch			
	3338		5788	
	B.P.C.	Spot	B.P.C.	Spot
S	82 (100 per cent)	70 (100 per cent)	116 (100 per cent)	102 (100 per cent)
269	80 (97·6)	68 (97·1)	118 (103·4)	98 (96·1)
12,753	80 (97·6)	70 (100)	116 (100)	100 (98·0)
13,897	80 (97·6)	68 (97·6)	114 (98·3)	98 (96·1)

For routine assay both methods can be satisfactorily applied using either a reference standard for protamine sulphate or for heparin. If a heparin standard is used, its capacity to neutralise protamine sulphate will have to be specified for the method which is to be used.

Acknowledgement. The authors wish to thank Mr. E. K. Maddison for his technical assistance in the development of this method.

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The paper was presented by MR. BIRKINSHAW.

THE OXIDATION OF EMULSIFIED AND SOLUBILISED BENZALDEHYDE

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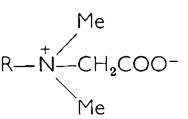
Received May 23, 1962

Carless and Nixon (1960) observed that when methyl linoleate was emulsified by cetomacrogol or potassium laurate, it oxidised at a rate greater than when it was solubilised. The rate of oxidation appeared to depend on the relative amounts of emulsified and solubilised oil present.

Subsequent work on the oxidation of aliphatic aldehydes in cetomacrogol solutions (Carless and Mitchell, 1962) supported this view. It was found that the oxidation rate depended on the saturation of the dispersion and not on the concentration of aldehyde and cetomacrogol, except in so far as these controlled the saturation. The degree of saturation of the dispersion was expressed as a Saturation Ratio (R) equal to X/Y, where X is the concentration of aldehyde present, and Y the concentration of aldehyde required to cause a saturated solution. Hence in a saturated solution $R = 1$, in an emulsion $R > 1$, whilst for a solution $R < 1$.

In an attempt to obtain further evidence we prepared an homologous series of pure ampholytic betaines of general formula



where R is a normal alkyl chain containing 8, 10, 12, 14, 16 and 18 carbon atoms. The rate of oxidation of benzaldehyde when solubilised and emulsified in aqueous solutions of these betaines, was investigated.

It must be emphasised that the term emulsion is used to cover all forms of dispersion in which oil is present in excess of its apparent solubility in aqueous soap solutions. Thus the term is used to cover not only the conventional definition of a dispersion containing two isotropic liquids, one being the continuous phase and the other the disperse phase, but also other dispersions of a more complicated nature containing an isotropic liquid or liquids with a liquid crystal phase.

Results and Discussion

Solubility curves of benzaldehyde in aqueous betaine solutions at 25° were determined by visual observation of the first turbidity. It was found that the apparent solubility of benzaldehyde decreased as the chain length of the soap increased. Examination of the dispersion under polarized light showed that the onset of turbidity marked the phase boundary between isotropic liquid and isotropic liquid plus a liquid crystal

phase. Winsor (1954) has emphasised that phase changes, similar to those noted above, occur at decreasing amphiphile concentration when the molecular weight of the soap is increased. Hyde, Langbridge and Lawrence (1954) have also stressed the importance of identifying the phases present. Failure to do this has led to confusion in interpreting solubility data.

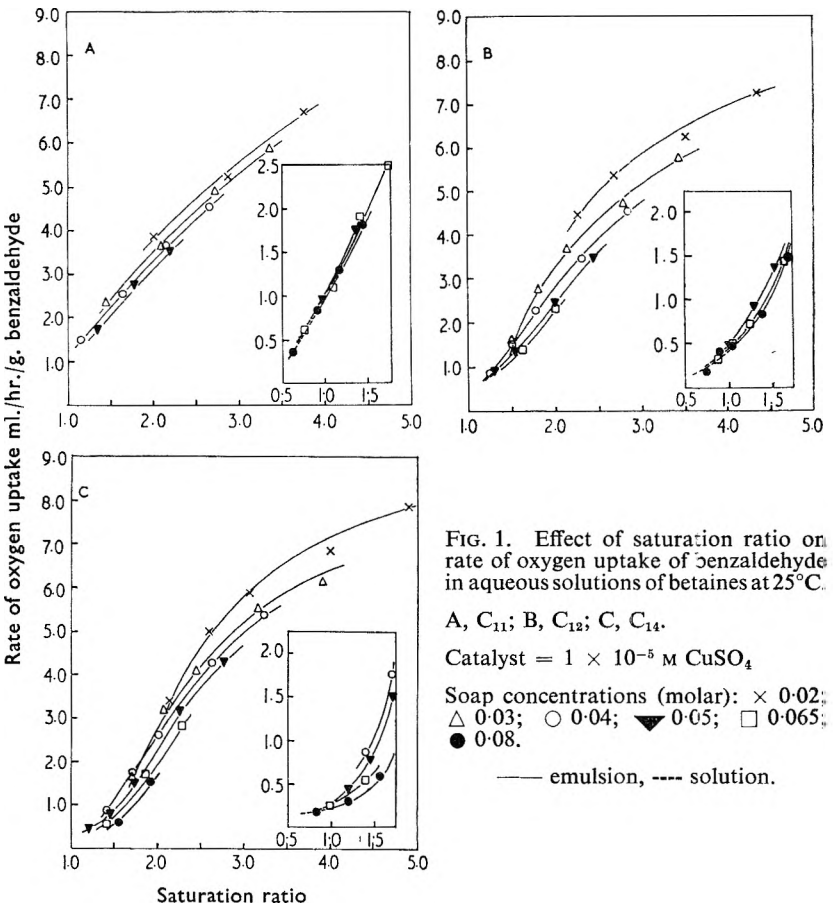


FIG. 1. Effect of saturation ratio on rate of oxygen uptake of benzaldehyde in aqueous solutions of betaines at 25°C.

A, C_{11}; B, C_{12}; C, C_{14}.

Catalyst = 1×10^{-5} M $CuSO_4$

Soap concentrations (molar): × 0·02; △ 0·03; ○ 0·04; ▼ 0·05; □ 0·065; ● 0·08.

—— emulsion, ---- solution.

The rates of oxygen uptake of benzaldehyde in water/betaine mixtures at 25°, were determined manometrically as described previously by Carless and Nixon (1957).

The relation between oxidation rate and saturation ratio (R) is shown in Fig. 1, A, B and C for betaines C_{11}, C_{12} and C_{14} respectively.

In all three instances, increase in soap concentration for any one particular value of R results in a slight, but consistent, lowering of the oxidation rate.

These results, together with others obtained for aldehydes dispersed in potassium laurate and cetrimide (Mitchell, 1960), show that R is not

applicable to these dispersions. Thus, even though the concept of a saturation ratio applies in the case of aldehydes dispersed in cetomacrogol, we believe that R is not of a fundamental nature.

Work in this department (Mulley, 1961) and elsewhere (Hyde, Langbridge and Lawrence, 1954; Dervichian, 1957) indicates that a variety of phases are likely to occur in soap-water-amphiphile systems, together with associated changes in the physical properties of the components.

A complete ternary phase diagram, such as that of potassium caprate–octanol–water (Dervichian, 1957), shows that it is possible, by varying the relative proportions of the three components, to:

(*a*) change the phase or phases in existence,

(*b*) alter the composition of the phases, although the type and number of these remain the same,

(*c*) alter the relative proportions of the phases present, even though the composition, type and number remain constant.

Thus, although various mixtures of components of a ternary system, giving rise to two or more phases, may possess the same saturation ratio, they will not contain, in the same proportions, similar phases of identical composition.

We feel, therefore, that only from a consideration of the ternary phase diagrams for these systems will it be possible to relate oxidation rates to the concentration and nature of the oil present.

Work is now in hand to obtain the ternary phase diagrams for the betaine–water–benzaldehyde systems, in those regions where oxidation rates are being studied.

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The paper was presented by MR. SWARBRICK.

Short Communication

SURFACE ACTIVITY OF A SERIES OF SYNTHETIC NON-IONIC DETERGENTS

By P. H. Elworthy and C. B. Macfarlane

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Received May 21, 1962

In contrast to ionic detergents, the non-ionic type provides an opportunity to alter the hydrophilic portion of the molecule by varying the number of ethylene oxide units in the polyoxyethylene chain; lengthening this chain increases the hydrophilic properties of the molecules. A series of compounds based on hexadecyl alcohol, of general formula:

$$Me(CH_2)_{15}(OCH_2CH_2)_nOH$$

where n varied from six to twenty-one, has been synthesised (Elworthy and Macfarlane, 1962). A study of their micellar shape and hydration has been made. In the present communication the critical micelle concentrations (CMC) and areas/molecule at the air/water interface are reported.

Apparatus and Method

Surface tension measurements were made using the Wilhelmy plate method (Harkins and Anderson, 1937). A depolished platinum plate, perimeter, $l = 6{\cdot}045$ cm., was suspended from the arm of a torsion balance, sensitivity $\pm 0{\cdot}2$ mg. The flask containing the solution was lifted by a rack and pinion device until the plate just touched the surface of the solution. The weight to lift the plate from the interior of the liquid to this point in the surface was determined. To ensure zero contact angle, all measurements were made by lifting the plate in this manner, and a saturated atmosphere was maintained above the solution. The difference between the weight of the plate in air and in the surface, W, is related to the surface tension, γ, by

$$\gamma = Wg/l$$

Measurements were made at 25° ± 0·01°. All glassware and the plate were cleaned with chromic acid, followed by thorough rinsing in distilled water. All flasks containing solutions were set aside for 3 hr., the flasks thoroughly drained, and fresh solutions made up in the same flasks. This procedure compensates for adsorption of solute by the glassware.

Results and Discussion

A check of the apparatus was made by measuring the surface tension of water, $\gamma = 72{\cdot}0$, 72·1 dynes/cm. (Harkins, 1959, gives 72·0 dynes/cm.), and of methanol, $\gamma = 22{\cdot}3$ (Timmermans, 1950, gives 22·2 dynes/cm.). Ageing effects were noted for all but the most concentrated solutions

studied; for a $9{\cdot}59 \times 10^{-7}$ mole/litre solution of hexaoxyethylene glycol monohexadecyl ether (Hn_6), the results were:

Time, hr.	0	1	2	3	4	5	7	9	10	26
γ, dynes/cm.	71·1	61·2	49·7	44·4	41·1	39·7	38·5	38·3	38·3	38·3

This effect may be due to a slow diffusion from the bulk to the surface, together with the possibility that considerable orientation of the molecules has to take place in the surface layer before equilibrium is reached.

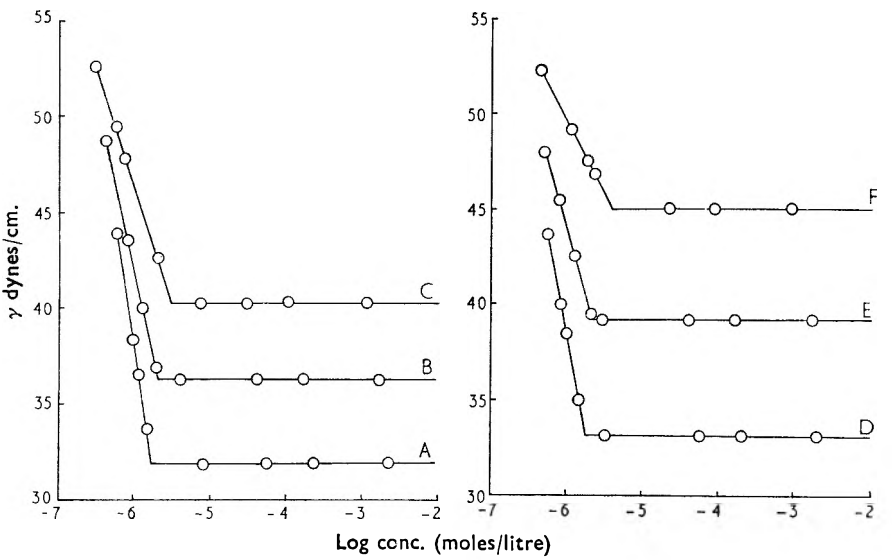

FIG. 1. Surface tension/log concentration plots for
$Me(CH_2)_{15}(OCH_2CH_2)_nOH$
A, $n = 6$, Hn_6; B, $n = 9$, Hn_9; C, $n = 15$, Hn_{15};
D, $n = 7$, Hn_7; E, $n = 12$, Hn_{12}; F, $n = 21$, Hn_{21}

In Figs. 1 and 2 the surface tensions of the detergents have been plotted against log concentration (moles/litre). From these graphs the CMCs were determined, and by using the simple form of the Gibbs' equation to calculate the surface excess, the areas/molecule were found.

Detergent	Hn_6	Hn_7	Hn_9	Hn_{12}	Hn_{15}	Hn_{21}
CMC, 10^6 moles/litre	$1{\cdot}6_6$	$1{\cdot}7_4$	$2{\cdot}0_9$	$2{\cdot}3_4$	$3{\cdot}0_9$	$3{\cdot}8_9$
Area/molecule (Å^2)	38	44	53	72	81	120
Area/(CH_2CH_2O) (Å^2)	6·3	6·3	5·9	6·0	5·4	5·7

It can be seen that a lengthening of the polyoxyethylene chain increases the CMC. This is a result of the increase in the hydrophilic properties of the molecule, which is in line with the fall in the micellar weight from $1{\cdot}27 \times 10^6$ for Hn_6 to $8{\cdot}2 \times 10^4$ for Hn_{21} (Elworthy and Macfarlane, 1962). The CMCs are represented by the equation:

$$\log \text{CMC} = -5{\cdot}93 + 0{\cdot}0245n$$

Hn_{21} falls in the range for cetomacrogol (n = 20 — 24), the CMC of which is higher than that of the synthetic material ($8 - 50 \times 10^{-6}$

moles/litre Hugo and Newton, 1960; Elworthy, 1960) which may be due to the polydisperse nature of cetomacrogol.

As the areas/molecule obtained are all larger than the cross sectional area of the hydrocarbon chain (20·5Å^2), it appears that the polyoxyethylene chain determines the area/molecule. Several workers have shown that the polyoxyethylene chain is considerably curled up in solution (Elworthy and Macfarlane, 1962; Rosch, 1961), and it seems likely that the chain is probably orientated in a position roughly parallel to the surface. The area/ethylene oxide unit *decreases* as the chain length *increases*. One end of the chain will be anchored to the surface by the hydrocarbon portion of the molecule, but the end remote from this point may be less strongly adsorbed at the surface, and may tend slightly away from it.

Acknowledgement. One of us (C.B.M.) thanks the Pharmaceutical Society of Great Britain for the award of a Lewis Edwards Memorial Scholarship.

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The paper was presented by MR. MACFARLANE.

THE CONTROLLED POTENTIAL REDUCTION OF CRYSTAL VIOLET AND BRILLIANT GREEN AT THE STIRRED MERCURY CATHODE

By C. G. Butler and (Mrs.) F. P. Martin

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Received May 23, 1962

The application of controlled potential coulometry to the electro-reduction of the triphenylmethane dyestuffs crystal violet and brilliant green is described below. The polarographic work by Kaye and Stone-hill (1952) indicated that, like the aminoacridines previously examined (Wilson, Butler, Ingle and Taylor, 1960), these were reduced in two stages each involving the uptake of one electron. The aim of the present work was to seek confirmation of this by direct measurement of the quantities of electricity involved in the reduction of a known amount of dyestuff.

Experimental Methods

The apparatus and techniques described by Wilson and others (1960) were employed. An electrolyte consisting of an aqueous buffer together with ethanol or dimethylformamide was depleted of reducible impurities by passing oxygen-free nitrogen through the solution and by prolonged electrolysis until the "background" current fell to a constant low value, usually not greater than 0·07 mA. To avoid changes in the composition of the mixed solvent, the gas was first passed through a wash bottle containing solvent of the same composition as that in the cell.

After adding a sample of the electro-reducible material, current-voltage diagrams were prepared using the method previously described. With ethanol as co-solvent, plots failed to show a sufficiently clear separation into the component steps to be of much value and 15 per cent dimethylformamide was used. Curves were then obtained showing two well defined steps but characterised by the presence of an anomalous fore-wave (Fig. 1a). Prolonged passing of nitrogen failed to remove this fore-wave but electrolysis for 20 min. at a potential of −700 mV was successful, and the subsequent current-voltage curve was found to be of normal appearance (Fig. 1b).

The appearance of the fore-wave was associated with the removal from the electrolysis cell of a few ml. of electrolyte in which the dye was dissolved before being returned to the cell. When this procedure was repeated omitting the dyestuff the fore-wave was still present in the buffer alone, and it is suggested that this phenomenon arises from the presence of some electro-reducible impurity in the dimethylformamide, reoxidised by contact with air. It had not been observed in parallel studies of aminoacridines in which ethanol was used as co-solvent.

The suggested procedure for coulometry in a solvent containing dimethylformamide is therefore as follows:

The mixed electrolyte (150 ml.) is submitted to electrolysis at the predetermined working potential in a stream of oxygen-free nitrogen until the background current had fallen to a low value. 5 ml. of the solution

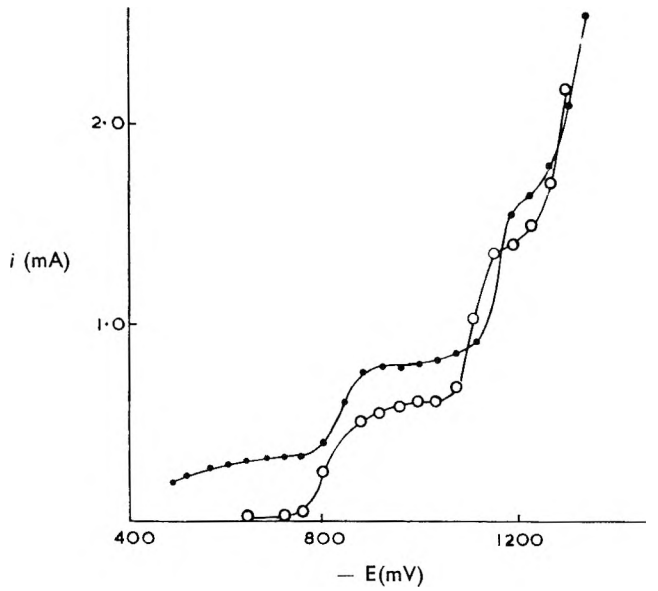


FIG. 1. Current - voltage curve of crystal violet in Sorensen's phosphate buffer (pH 7·4) (a, ●——● showing anomalous fore-wave; b, ○——○ after preliminary electrolysis at 700 mV) containing 15 per cent v/v of dimethylformamide.

is withdrawn, used to dissolve the weighed sample of dye and returned to the cell. Electrolysis is carried out under nitrogen for a further 20 min. at −700 mV to remove impurities. The potential is then adjusted to the appropriate value (−1000 mV for crystal violet), and decay of current with time observed. A typical current-time plot is shown in Fig. 2; from this a note was made of the initial current (I_0) and half-time ($t_{\frac{1}{2}}$) of the reaction. Substitution in the equation

$$Q = \frac{I_0 \times t_{\frac{1}{2}} \times 60}{0{\cdot}693} \text{ coulombs, where } I_0 \text{ is in amperes and } t_{\frac{1}{2}} \text{ in min.,}$$

gives the quantity of electricity which would be required for the reduction of the entire sample. The n value is calculated from

$$n = \frac{QM}{96{\cdot}5\ W}$$

where M = molecular weight of the dye
W = wt. of sample in mg.

Experimental Results

Determination of the n value for the reduction of crystal violet at −1000 mV (corresponding to the first step of the polarographic wave) yielded the data in Table I.

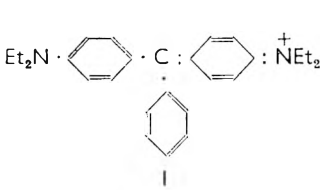
TABLE I

N-VALUES FOR CRYSTAL VIOLET (FIRST STEP OF REDUCTION)

Wt. of sample mg.	I_0 mA	$t_{\frac{1}{2}}$ min.	n value
1·9	0·48	11·1	1 027
2·8	0·485	15·7	0 99
2·55	0·54	13·3	1 028
3·1	0·654	14·5	1 14
3·0	0·52	19·2	1 18
2·5	0·448	16·2	1 06
2·5	0·425	17·8	1 10
2·5	0·44	18·5	1 186

The n-value of 1 confirms the conclusions of Kaye and Stonehill (1952).

With brilliant green at slightly alkaline pH values the dyestuff was slowly converted from the coloured ionic form (I) to the colourless unionised and non-reducible form (II).



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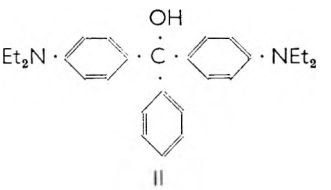

II

The colour of the solution slowly faded before electro-reduction could be commenced, and experimental n-values were in all cases less than 1 (Table II).

TABLE II

N-VALUES FOR BRILLIANT GREEN (FIRST STEP) pH 7·4*

Wt. of sample mg.	I_0 mA	$t_{\frac{1}{2}}$ min.	n value
2·1	0·30	9·7	0·63
1·7	0·355	9·2	0·83
1·95	0·38	6·8	0·57
3·0	0·325	11·7	0·55
3·0	0·35	31·8	0·62
3·0	0·185	23·1	0·61

When the reduction was investigated at pH 5·6* there was no noticeable fading of the colour of the solution prior to electrolysis, but separation of the current-voltage curve into two steps was insufficiently well marked to allow a satisfactory coulometric investigation of the first step. This is in accordance with Kaye and Stonehill (1952) who observed that the two steps of the polarographic wave were well separated only in alkaline solutions. Coulometric determinations were therefore carried out at a

* pH values of aqueous buffers used in the mixtures.

potential of −1,200 mV, corresponding to the top of the second wave; these indicated a n-value of 2.

It may be concluded that the triphenylmethane dyestuffs crystal violet and brilliant green are reduced at the stirred mercury cathode in two stages each involving one electron. The instability of brilliant green in alkaline solution has hindered a direct determination of the n-value corresponding to the first step, but the overall reduction (2 steps) has been shown to involve two electrons. In the case of crystal violet which is reduced in two steps of equal height, the first has been shown to correspond to the uptake of one electron.

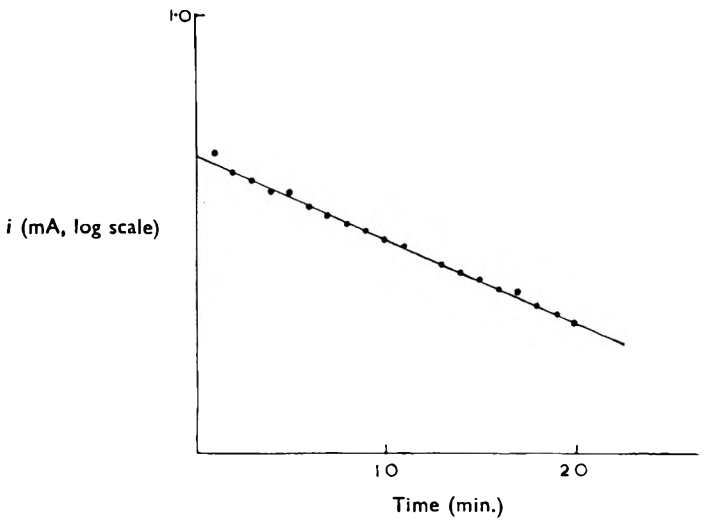

FIG. 2. Current decay plot for crystal violet (control potential − 1000 mV *vs* S.C.E.)

The conclusions reached by Kaye and Stonehill (1952) from polarographic data have thus been confirmed and may be of significance in view of Kaye's (1950) theory that bacteriostasis by certain dyes is related to free radical formation.

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The paper was presented by DR. BUTLER.

STUDIES ON *DATURA LEICHHARDTII* MUELL. EX BENTH.

PART II. ALKALOIDAL CONSTITUENTS

BY W. C. EVANS AND N. A. STEVENSON*

From the University, Nottingham

Received May 9, 1962

The variation in the proportion of hyoscyamine and hyoscine in the aerial parts of *Datura leichhardtii* has been investigated. From the roots, the alkaloids 7-hydroxy-3,6-ditigloyloxytropane, 3α-tigloyloxytropane and meteloidine have been isolated.

BRIEF reports are available on the poisonous nature of *Datura leichhardtii* Muell. ex Benth. towards sheep and other animals (Hurst, 1942; Gardner and Bennetts, 1953). The presence of alkaloids in the plant (Sievers, 1921) has been noted, but no investigation into the composition of the alkaloidal mixture has been made. An investigation on this point, together with an ontogenetical study of alkaloid production, was therefore undertaken.

EXPERIMENTAL

The source of plant material is as indicated in Part I (Evans and Stevenson, 1962).

Plants were raised from seed in a temperate greenhouse, and to prevent early ageing were exflorated until a height of about 45 cm. was attained. Flower buds were then allowed to develop until the corollas protruded through the calyces, when the aerial parts and roots were separately harvested and dried at 65°.

Alkaloids of the Aerial Parts

The powdered, dried leaves and stems gave a positive Vitali reaction and when analysed for tropic acid esters by the method of Colby and Beal (1952) gave an alkaloidal content of approximately 0·1 per cent. The powder (200 g.) was moistened with water (120 ml.), allowed to stand overnight and then triturated with calcium hydroxide (20 g.). The mixture was shaken (1 hr.) with solvent ether (1·5 litres), the supernatant liquid decanted and the marc further percolated with ether (2·5 litres). Ether extracts were concentrated and passed through a column of purified kieselguhr (30 g.) loaded with N sulphuric acid (20 ml.). Pigments were removed by elution with ether and the alkaloids were recovered with chloroform/ammonia.†

The basic residue thus obtained was chromatographed on kieselguhr (30 g.) loaded with phosphate buffer (pH 6) (20 ml.) as described by Evans and Partridge (1952). Ether (500 ml.) yielded (−)-hyoscine

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† The lower layer produced by shaking chloroform (500 ml.) with Strong Solution of Ammonia B.P. (20 ml.).

(98 mg.), picrate m.p. 187° giving no depression on admixture with an authentic specimen. Chloroform (28 ml.) gave a mixture of bases (57 mg.) which could not further be separated: the picrate had m.p. 158–160° after extensive recrystallisation. Chloroform (a further 175 ml.) yielded hyoscyamine (144 mg.), picrate m.p. 164–165° giving no depression with an authentic sample. Finally, treatment of the column with chloroform/ammonia yielded an unidentified base (11 mg.) whose picrate had m.p. 228° after extensive recrystallisation.

Paper chromatography of extracts from other samples of *D. leichhardtii* showed that hyoscyamine and hyoscine were the principal alkaloids. Quantitative analyses (Evans and Partridge, 1952; Evans and Than, 1962) are shown in Table I.

TABLE I

ALKALOIDS OF AERIAL PARTS OF *DATURA LEICHHARDTII* MUELL. EX BENTH.

Sample*	Total alkaloids in dried sample per cent (as hyoscyamine)	Hyoscine as per cent total alkaloid where applicable
Seedlings, 2–3 days after cotyledons appeared ..	—	Principal alkaloid had R_F value = hyoscyamine; some hyoscine and traces of two bases of low R_F value.
Seedings, 1 week after emergence of cotyledons	—	Hyoscine and hyoscyamine spots of about equal magnitude. Traces of other bases of lower R_F value.
Seedlings, 2 weeks old‡	0·13†	55
Seedings, 4 weeks old	0·10	53
Young plants, 6 weeks old	0·16	47
Plants, 8 weeks old	0·09	46
Plants, 10 weeks old, average height 30 cm., 1 capsule	0·14	29
Plants, 12 weeks old, average height 35 cm., 2–3 young capsules	0·13	31
Plants, 14 weeks old, average height 35 cm., 2–3 mature capsules	0·08	29
Plants, analysed at end of season after removal of ripe fruits	0·02	7
Plants with young capsules. Raised in 1959 ..	0·04	20
Dried, Australian grown sample with fruits, 1952	0·03	20
Ripe seeds	0·16	28

* Raised in 1960, except where indicated.
† Method of Evans and Partridge (1952); Evans and Than (1962).
‡ Composite sample from different batches.

Ontogenetic Production of Alkaloids

Seedlings and plants of varying ages were examined for alkaloidal content at regular intervals. Analyses are recorded in Table I. The separated alkaloids of the 4, 6 and 8 week-old plants were combined and identified by the preparation of their picrates and measurement of their R_F values by paper chromatography. The alkaloids were similarly identified in each subsequent sample.

Alkaloids of the Roots

Roots (100 g.) were extracted as described above for the aerial parts and then transferred with light petroleum to a column of purified kieselguhr (25 g.) loaded with 0·5M phosphate buffer solution (15 ml.), pH 6·6.

Light petroleum (b.p. 40–60°) (25 ml.) afforded an oil (53 mg.) which, after chromatography a second time gave 7-hydroxy-3,6-ditigloyloxytropane, picrate m.p. 177° undepressed on admixture with an authentic specimen. Ether (148 ml.) yielded a material (5·5 mg.) with an R_F value equivalent to that of tigloidine; the picrate m.p. 210° raised to 224–230° (decomp.) after four recrystallisations had m.p. 238° when admixed with a sample of tigloidine picrate, m.p. 240°. Ether (288 ml.) gave 3α-tigloxytropane (13 mg.), picrate m.p. 178–179° giving no depression on admixture with an authentic sample. Chloroform (38 ml.) yielded meteloidine (64 mg.), picrate m.p. 178–179° undepressed on admixture with an authentic sample. Chloroform (a further 185 ml.) gave unidentified bases (11 mg.).

Final elution of the column with chloroform/ammonia gave a small quantity of basic material with a low R_F value.

Discussion

The composition of the alkaloids from the aerial shoots of *D. leichhardtii* is similar to that in *D. stramonium*. In the flowering and young fruiting plants, hyoscyamine and hyoscine, the principal alkaloids, occur

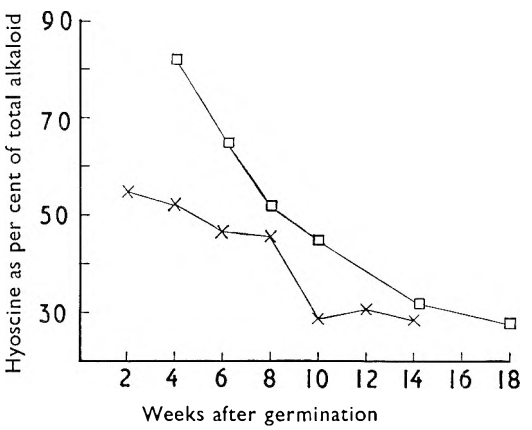

Fig. 2. Variation of the proportion of hyoscine in the total alkaloids of *Datura* spp. with age of plants. ×——×, *D. leichhardtii*; □——□, *D. stramonium* var. *godronii* (from Evans and Partridge, 1953).

in the ratio of about 2:1, whereas in most *Datura* species examined to date, hyoscine is the main alkaloid of the aerial parts. In old plants the ratio of hyoscyamine to hyoscine may rise to 9:1. This increase during development (Table I) is similar to that observed in *D. stramonium* (Hegnauer, 1951; Evans and Partridge, 1953) (see Fig. 1) and also agrees with the conclusions reached for *D. stramonium* on the basis of isotopic feeding experiments (Romeike and Fodor, 1960 and references there cited). Surprisingly, however, we have been able to find neither hyoscyamine nor hyoscine in the roots of mature *D. leichhardtii* plants. Instead the principal alkaloids here are 7-hydroxy-3,6-ditigloyloxytropane, 3α-tigloyloxytropane, meteloidine and possibly tigloidine, although the presence of

tropyl esters in small quantity cannot be excluded. Further experiments are planned to extend our observations on root-alkaloids to all ages of the plant and over a range of environmental conditions.

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Discussion

The paper was presented by MR. STEVENSON. The following points were made in the discussion.

The total alkaloid content was relatively low in this species, which therefore did not have commercial value. Up to 0·04 per cent of tropyl esters had been found in the roots. The third base from the aerial parts gave a picrate of m.p. 228° and was thought to be noratropine. Many factors were involved in the chromatography of the bases and the conditions had to be adjusted empirically. The first hybrids produced a high yield of alkaloids. Diurnal variation was not studied: the plants were collected in the morning.

THE PARTICLE SIZE DISTRIBUTION OF MARBLE ON WET BALL MILLING

Effect of the Solid : Liquid Ratio

By M. I. Barnett and K. C. James

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Received May 18, 1962

An investigation has been made of a wet ball milling procedure which depends on the apparent viscosity of the charge increasing during the operation until the balls are held by the charge, and circulate with the mill. By varying the solid : liquid ratio of the charges the mean particle size of the product was changed, and shown to be a linear function of the proportion of solid in the charge. The effect of changing the ball size is discussed.

The critical speed N_c of a ball mill is the rotational velocity at which the balls cease to grind, and are carried around with the mill by centrifugal force. In wet milling the viscosity of the liquid affects the critical speed, since it impedes the fall of the balls, and brings about critical conditions below those predicted by the generally accepted formula

$$N_c = KD^{-\frac{1}{2}}$$

where D is the diameter of the mill, and K is a constant depending on the units in which D is measured. As the particle size of the charge is reduced, the apparent viscosity of the suspension increases, so that critical conditions can result after milling for a given time, even though the rotational velocity of the mill was below critical at the beginning of the operation. Coghill and De Vaney (1937) have suggested this sequence as the cause of an observed fall in the rate of comminution with time.

Frequently it is important that a pharmaceutical material be presented within a narrow particle size range. Thus it was considered that a study of the behaviour described above might reveal a self limiting method whereby milling would stop to give a pre-determined and reproducible particle size range.

Experimental and Results

Materials

Marble chips of chemical reagent quality were ground in an end runner mill, and material which passed a number 10 but not a number 22 sieve was used for milling. A saturated aqueous solution of calcium carbonate was used as the liquid phase in the ball millings, and as a diluent in the sampling procedure; this was prepared from the fines. To obtain a satisfactory medium for the determination of particle size distribution after milling, Dispersol T (I.C.I. Ltd.) and Antifoam Emulsion A (Midland Silicones) were used.

Milling Procedure

Milling was carried out in a 16 oz. cylindrical glass pot of diameter 2·5 in. using stainless steel balls, which together with the voids filled half of the pot. The same weight of balls was used in every experiment, and the total charge of marble and liquid was maintained at 200 g. This together with the balls occupied 65 per cent of the volume of the pot. A constant velocity of 140 r.p.m. was used throughout and milling was continued until every ball was circulating with the mill.

A series of millings of marble chips (10/22 mesh) were made in a saturated aqueous solution of calcium carbonate using $\frac{3}{8}$ in. balls, and varying the solid content of the charge from 0·375 to 0·625. Experiments were confined within these values since charges containing less than 0·375 of solid took over a week to reach limiting conditions, while solids in excess of 0·625 were not reduced completely and yielded a proportion of unmilled material.

In repeating the procedure with $\frac{1}{2}$ in. balls, their greater mass enabled them to mill charges containing a larger proportion of solid. The range used was from 0·3 to 0·7. With the $\frac{1}{4}$ in. balls, the upper limit of solid was 0·425 so that weaker charges had to be examined. Charges containing less than 0·25 of solid took more than five days to reach limiting conditions, consequently 0·25 was used as the lower limit.

Sampling

The sampling procedure was a development of a technique by Ellis (1953). The balls were strained off through a coarse sieve, and the milled material suspended in 1 litre of saturated calcium carbonate solution containing 1 per cent Dispersol T, to stop aggregation of the particles, and 1 part per million of Antifoam Emulsion A. The suspension was transferred to a beaker and stirred mechanically at a speed such that the solid was uniformly suspended, and no air was introduced. After 10 min. 0·1 ml. was withdrawn (the pipette being gradually raised in the beaker during the operation) and adjusted to 100 ml. After shaking for a few min. one drop was transferred to a Thoma haemocytometer slide and the particles counted in the usual manner. First double image diameters of the particles, as described by Timbrell (1962) were measured using a Timbrell Double Image Micrometer and Particle Size Analyser. This instrument and procedure have been described elsewhere by Barnett and Timbrell (1962).

To show that there was no preferred orientation of the particles, the first double image diameters of 1,000 particles were measured, then the field moved through 90 degrees and the new diameters of the same particles measured. There was no significant difference between the two sets of measurements.

It was found adequate to measure 1,500 particles and to place these in size groups since no significant difference in distribution or average diameter was observed in the histograms derived from a batch of 1,500 particles or from one of 3,000 particles.

For each experiment six samples were taken, and 250 particles measured in each sample. The size distributions of the particles obtained with $\frac{3}{8}$ in. balls are shown in Table I.

TABLE I

VARIATIONS IN PARTICLE SIZE DISTRIBUTION WITH CHANGES OF WEIGHT RATIO OF SOLID IN CHARGE USING $\frac{3}{8}$ IN. STAINLESS STEEL BALLS

Size Group (μ)	Number of particles in each size group for the following values of $\frac{\text{weight of solid}}{\text{weight of solid and liquid}}$					
	0·375	0·400	0·425	0·500	0·575	0·625
1–2	43	5	15	19	—	—
2–3	815	405	647	237	201	157
3–4	446	836	582	723	942	835
4–5	127	169	130	236	172	234
5–6	31	29	50	114	51	82
6–7	9	15	23	61	56	43
8–10	16	15	22	57	64	93
10–15	8	11	13	28	22	33
15–25	3	7	10	10	3	14
25–50	0	0	0	0	8	8

DISCUSSION

The most obvious parameter of particle size distribution with which to compare the results would appear to be the arithmetic mean of all the measurements, that is, the mean diameter $d_{av} = \frac{\Sigma nd}{\Sigma n}$. However, since the fine particles are more numerous than the coarse particles, this would represent only a small portion of the total weight of the mass. The mean volume diameter, $d_v = \sqrt[3]{\frac{\Sigma nd^3}{\Sigma n}}$, and the weight mean diameter $d_w = \frac{\Sigma nd^4}{\Sigma nd^3}$ are more representative of the bulk of the mass.

The mean volume diameter has been defined by Dallavalle (1948) as the diameter whose corresponding volume divided into the total volume, gives the total number of particles. Perrott and Kinney (1923) have defined the weight mean diameter as the diameter d_w, such that half the weight of the sample consists of particles of diameter d_w or less, and half of the particles of diameter d_w or more. The two means coincide only in normal distributions, and the difference between them is a measure of the degree of skew.

The variations of the three means described above with weight fraction of solid in the charge are plotted in Fig. 1. and in each case a linear relationship obtains. Examination of Table I shows that these changes are not due to a shift of the maximum from the 2 to 4 μ region, but to an increase in the number of particles occurring in the higher size groups. This is confirmed from Fig. 1 since the difference between d_v and d_w increases with weight fraction of solid, indicating an increase in skew in the distribution curves.

Both the volume and the mass of the balls are altered by changing their size, and these affect the final particle size in opposite ways. It is well established that with smaller balls there are more points of contact, and the milling is more efficient. In the procedure described, however, the heavier the balls, the longer will they resist the retention by the charge, and once again a finer product will result. Fig. 2 shows the variation of weight mean diameter with weight fraction of solid for the three ball sizes, and shows there is no correlation between ball size, and the particle size of the product. It is suggested that the effects of mass and volume,

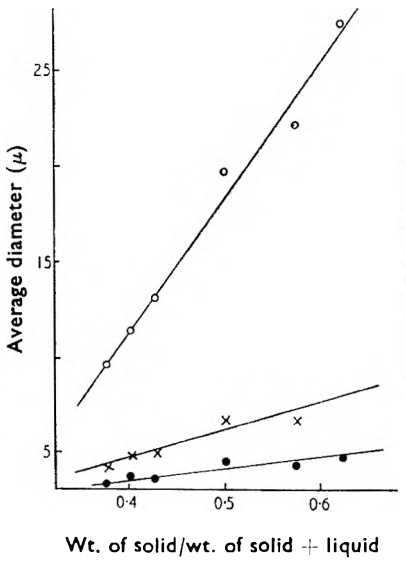

FIG. 1. Effect of solid content of charge on particle size of product

● Mean diameter (d_{av})
× Mean volume diameter (d_v)
○ Weight mean diameter (d_w)

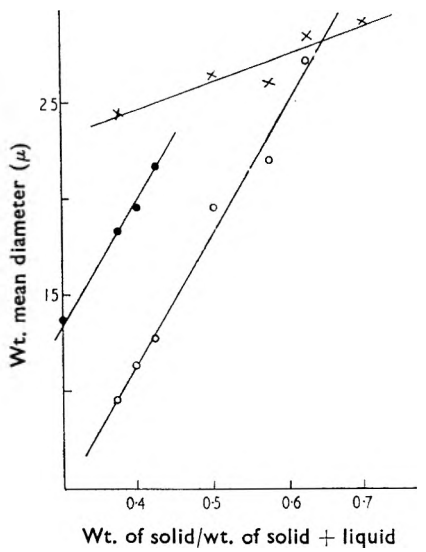

FIG. 2, Effect of ball size on weight mean diameters.

● ¼ in. ○ ⅜ in. × ½ in.

working against each other are responsible for this lack of correlation, so that with the ½ in. balls the disadvantage of having a larger volume than the ⅜ in. balls is greater than the advantage of their heavier mass, while with the ¼ in. balls the weight disadvantage exceeds the advantage gained from having a smaller volume.

It is apparent from the results that the average particle size of the milled product can be influenced by using the process that has been described. Further, since the relationship between average size and weight fraction of solid is linear, then if two millings be carried out with different solid : liquid ratios, all other conditions remaining constant, the mean particle size produced by any other ratio can be predicted. Alternatively a solid : liquid ratio necessary for a required average particle size may be calculated.

Acknowledgements. We thank Dr. V. Timbrell for his advice in the particle size measurements, and Imperial Chemical Industries Ltd., for the gift of samples of Dispersol T.

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Discussion

The paper was presented by MR. BARNETT. The following points were made in the discussion.

Marble was selected for its insolubility in water. The criteria for selecting the weight of balls and charge were the half-way filling of the mill with the balls, which were then adjusted to a specific weight, and sufficient charge, also adjusted to a specific weight, to fill the void spaces and to cover the balls. The endpoint at which the balls ceased to move was sharp. Errors within and between observers making the particle analysis were low and smaller than usual in microscopical analysis.

Short Communication

DETERMINATION OF TRICHLOROETHYL PHOSPHATE IN PHARMACEUTICAL PREPARATIONS

By P. F. G. Boon

From the Analytical Department, Glaxo Laboratories Ltd., Greenford, Middlesex

Received May 23, 1962

Introduction of the monophosphoric ester of trichloroethanol as a hypnotic made it necessary to develop analytical methods for determining the ester in the presence of its decomposition products. These may arise by ester hydrolysis to yield trichloroethanol followed (or preceded) by other changes due for example to attack on the trichloromethyl part of the molecule. As would be expected, aqueous solutions which have decomposed as a result of prolonged or unsatisfactory storage contain chloride and phosphate ions. The presence of formaldehyde has been demonstrated in autoclaved aqueous solutions.

Published methods for determining trichloroethanol and closely related compounds were examined (Marshall and Owens, 1954; Seto and Schultze, 1956; Rehm and Mader, 1957; Friedman and Cooper, 1958; Archer and Haugas, 1960). None was found suitable for the esterified alcohol.

Enzymatic hydrolysis of the phosphate link (Boon, 1960) worked satisfactorily and the liberated trichloroethanol was isolated by steam distillation, then estimated by the method of Marshall and Owens (1954). Although satisfactory, this procedure was rather cumbersome for routine use. Halogenated compounds have been determined by alkaline hydrolysis followed by titration of the chloride ion thus produced. Application of this method was facilitated by the observation that trichloroethyl phosphate could be extracted by amyl alcohol from acidified aqueous solutions. The combined chlorine proved somewhat resistant to hydrolysis, and required treatment with 2N alcoholic potassium hydroxide for 2 hr. at 120°.

Proposed Volumetric Method

Transfer to a 100 ml. separator, sufficient sample to contain about 120 mg. of trichloroethyl phosphate. Add water (15 ml.) and swirl to dissolve. Adjust to about pH 9 with N sodium hydroxide, add ether (15 ml.), stopper, shake for 1 min., and set aside for 2 min. Transfer the lower aqueous layer to a second separator. Wash the ether with water (1 ml.). Add the washings to the aqueous phase, and reject the ether. Any free trichloroethanol is thus removed from the sample. Add dilute sulphuric acid (B.P. reagent) (2·5 ml.) and amyl alcohol (10 ml.) to the aqueous phase. Stopper, shake for 1 min. and set aside until the phases separate. Transfer the lower aqueous layer to a second separator, and extract with three further 10 ml. portions of amyl alcohol. Combine the amyl alcohol extracts; reject any aqueous phase present. Transfer

to a dry 50 ml. volumetric flask, dilute to 50 ml. with amyl alcohol, and mix. Place a 10·0 ml. aliquot in a 20 ml. ampoule. Add 2N alcoholic potassium hydroxide (10 ml.) to the ampoule, and seal. Autoclave the ampoule for 2 hr. at 15 lb. per sq. in. Cool, and transfer the contents to

TABLE I

COMPARISON OF VOLUMETRIC AND ENZYMATIC METHODS

Autoclaving time min.	Trichloroethyl phosphate content per cent w/v	
	Proposed method	Enzyme method
0	4·13	4·3
8	3·55	3·5
18	2·63	2·7
30	1·31	1·4

a 100 ml. conical flask, washing in with dilute nitric acid (B.P. reagent) (20 ml.). Add ferric ammonium sulphate solution (B.P. reagent) (4 ml.) and 0·1N silver nitrate (5·00 ml.). Titrate with 0·2N ammonium thiocyanate solution.

TABLE II

RESULTS OBTAINED BY THE VOLUMETRIC METHOD

Preparation	Trichloroethyl phosphate	
	Calculated	Found
Syrup	7·0 per cent w/v	6·9 per cent w/v
Syrup	14·0 per cent w/v	13·8 per cent w/v
Tablet	500 mg.	493 mg.
Effervescent tablet ..	500 mg.	490 mg.

1·0 ml. of 0·02N silver nitrate is equivalent to 1·676 mg. of trichloroethyl monosodium phosphate.

The determination of solid trichloroethyl monosodium phosphate by this method gave values within 1 per cent of those obtained by sodium carbonate fusion.

The recommended method determines chlorine-containing materials which can be extracted from acid solution by amyl alcohol. Whilst it eliminates at least 95 per cent of free trichloroethanol and sodium chloride, other possible decomposition products such as trichloroacetic acid and dichloroacetaldehyde are not removed in the course of the assay, and lead to high halogen values. As a check on whether the assay does indeed accurately define the content of trichloroethanol monosodium phosphate the determination was repeated using an alkaline phosphatase hydrolysis (Boon, 1960). Free trichloroethanol was then assayed by the more specific method of Marshall and Owens (1954).

When applied to substrates of 0·1 and 0·2 mg. of monosodium trichloroethyl phosphate, recoveries of trichloroethanol of 98·5 and 95·3 per cent respectively, resulted.

Aqueous solutions of the ester, deliberately decomposed to different extents by autoclaving, were assayed by both methods, and Table I

shows the results obtained. The agreement between the two is taken as confirmation that the recommended method is satisfactorily specific.

Some results obtained by the method are shown in Table II. Aqueous preparations were freshly prepared from accurately weighed quantities of trichloroethyl phosphate (monosodium salt).

Acknowledgements. The author thanks Mr. A. Hibbert for assistance with the practical work, and Mr. W. H. C. Shaw for helpful comments.

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The paper was presented by Mr. Boon.

THE EFFECT OF A SULPHATED POLYSACCHARIDE ON THE ACIDITY AND VOLUME OF HISTAMINE-STIMULATED GASTRIC SECRETION IN THE GUINEA-PIG

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Received May 14, 1962

Degraded carrageenan administered in aqueous solution via an oesophageal tube to guinea-pigs twice daily and also freely available as drinking fluid, for 8 to 14 days, causes a 50 per cent reduction in the volume and acidity of histamine-stimulated gastric juice. This could be an important factor in the protection afforded by carrageenan against histamine-induced duodenal ulceration.

Degraded carrageenan, a sulphated polysaccharide, forms a complex with the mucoprotein of acidified gastric mucus. *In vitro*, experiments have shown that this material retards the diffusion of pepsin through the mucus layer (Anderson, 1961). The possibility that the mucoprotein complex or even degraded carrageenan itself might reduce gastric secretion arose during the course of clinical investigations on this substance. Preliminary studies in the guinea-pig indicated that degraded carrageenan administered over a 36 hr. period caused a 40 to 50 per cent reduction in the acidity of histamine-stimulated gastric juice. We have now administered degraded carrageenan over a longer period, and have studied the effects on both the volume and acidity of histamine-stimulated secretion.

Methods

Animals were maintained on an ordinary cube and cabbage diet. Drinking fluid was supplied freely at all times, control animals receiving water, experimental animals receiving a 3 per cent aqueous solution of degraded carrageenan which was readily accepted. Degraded carrageenan (20 per cent solution) was also given at intervals via an oesophageal tube, the final dose coinciding with the removal of all food from the cages some 15 hr. before administering histamine and collecting the gastric juices. The histamine preparation used was the acid phosphate suspended in a beeswax : arachis oil vehicle (10 mg. histamine acid phosphate per ml.). Injections were given intramuscularly usually at 9 a.m. in doses of 10 mg. histamine acid phosphate per kg. One and a half hr. later, a stomach-tube was introduced via the oesophagus, the gastric contents were removed, the volumes recorded and the free and total acidities measured by titration with 0·04N NaOH, using Topfer's reagent and phenolphthalein as indicators.

In the first experiment, 11 small female guinea-pigs (350 g. body weight) were given 5 ml. of 20 per cent degraded carrageenan twice daily by stomach tube for 8 days, in addition to free access to a 3 per cent solution as drinking fluid. The volume and acidity of the histamine-stimulated

gastric juices were examined at the start of the experiment, at the end of the 8 days treatment and finally after a further 14 days during which time no degraded carrageenan was given. Four animals were killed for histological examination of the stomach.

In the second experiment, 8 male Albino guinea-pigs of larger size (600 g.) were used. After obtaining control histamine-stimulated gastric juices, the animals received the same treatment as in the first experiment but over 14 days. Histamine-stimulated juices were then collected from 4 animals in the group, and from the remainder at the end of a further 7 days, during which time no degraded carrageenan was given.

Results

The results are shown in Tables I and II. After administering degraded carrageenan for 8 days there was approximately a 50 per cent reduction both in the volume and acidity of the gastric juices. At the end of 2

TABLE I

EFFECT OF 8 AND 14 DAYS ADMINISTRATION OF DEGRADED CARRAGEENAN ON HISTAMINE-STIMULATED GASTRIC SECRETION

	Gastric acidity (ml. 0·1N HCl/100 ml.)				Volume of juice (ml.)	
	No. of guinea-pigs	Range Free acid (FA)	Range Total acid (TA)	Average FA/TA	Total	Average per animal
	Experiment I—Small animals (350 g.)					
Before carrageenan	11	99–143	110–152	124/134	50·7	4·6
After 8 days carrageenan	9	24–97	42–109	58/72	19·3	2·1
After 2 weeks off carrageenan	7	100–128	115–150	110/129	33·5	4·8
	Experiment II—Large animals (600 g.)					
Before carrageenan	8	47–129	90–136	105/117	69·5	8·6
After 14 days carrageenan	4	38–80	50–98	58/81	14·4	3·6
After 14 days carrageenan and off for 7 days	4	116–123	126–131	119/128	42·5	10·6

weeks, during which time the animals received no further amounts of degraded carrageenan, the volume and acidity of the histamine-stimulated juices had returned to normal control values. When carrageenan was given over 14 days to larger animals, secreting larger volumes of juice, a similar reduction in the histamine-stimulated juices was obtained. A return to normal values took place when the animals had been 7 days without carrageenan. No histological changes were noted in the gastric mucosa after 8 or 14 days treatment with carrageenan.

Discussion

Recently it has been shown that degraded carrageenan imparts some protection against histamine-induced gastro-duodenal ulceration in both the dog (Houck, Bayana and Lee, 1960) and guinea-pig (Anderson and Watt, 1959). Factors believed to be concerned in the protective mechanism and so far studied are the inhibition of peptic digestion and the the enhancing of the protective function of the mucus lining the mucosa.

The above findings isolate another factor and indicate that degraded carrageenan, either by itself or in complexed form, may reduce both the volume and acidity of the gastric secretion by as much as 50 per cent.

TABLE II

RESULTS FOR INDIVIDUAL ANIMALS IN THE GROUP OF 4 BEFORE AND AFTER RECEIVING CARRAGEENAN FOR 14 DAYS

	Volume (ml.)		Histamine-stimulated gastric juice Free Acid/Total Acid (ml. 0·1N HCl/100 ml. of gastric juice)	
	Before	After	Before	After
	19	4·5	128/135	67/85
	13	5·4	129/136	80/92
	2	3·5	84/95	48/98
	8	1·0	126/133	38/50
Totals	42	14·4	—	—
Mean per animal	10·5	3·6	117/125	58/81

This is likely to be an important factor in the prevention of histamine-induced ulceration, particularly duodenal ulceration which is frequently associated with, and attributed to, the hypersecretion of highly acid gastric juice.

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The paper was presented by DR. WATT.

Short Communication

THE POLAROGRAPHIC ASSAY OF STREPTOMYCIN

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The use of polarography to assay streptomycin has received limited attention. Levy, Schwed and Sackett (1946) have reported on its application at streptomycin concentrations of greater than 200 units/ml. using partially purified material. Bricker and Vail (1951) have used it to study the alkaline degradation of streptomycin and have concluded that the independent determination of streptomycin and mannosido-streptomycin in mixtures of the two is impracticable. The existence of two tautomeric forms of streptomycin has been demonstrated by this technique Heuser, Dolliver and Stiller (1953), whilst Tsukamoto and Tachi (1952) have shown that the height of the reduction wave increased with pH increase, and recommend N sodium hydroxide as a base electrolyte. Much of the work on the polarography of streptomycin is summarised by Brezina and Zuman (1958). The present paper describes a development of the polarographic method mainly for the assay of streptomycin fermenter broth and associated recovery stages.

Experimental and results

Assay of Solutions of Pure Streptomycin Sulphate (International Standard of 780 units/mg.)

The work described was carried out using a Tinsley Mark 19 pen recording polarograph, with mercury capillaries of drop times between 2 and 3 sec. at a 50 cm. height. As an alternative to tetramethylammonium hydroxide used by Levy and his co-workers (1946) the experiments described below used lithium hydroxide as base electrolyte (Wise, unpublished). Its advantage over sodium hydroxide (Tsukamoto and Tachi, 1952) was that it had a more negative half-wave potential.

Initial experiments with pure streptomycin showed that polarograms from solutions with concentrations below 200 units/ml. were difficult to measure. This difficulty was caused by the poor resolution of the slopes due to the residual, limiting and diffusion currents, and also, in less pure solutions, by additional reduction waves. Use of the derivative circuit reduced the overall sensitivity but improved the resolution so that it became possible to measure polarograms from solutions as dilute as 10 units/ml. The streptomycin response moreover was rectilinear and proportional up to at least 300 units/ml. Variation of the lithium hydroxide concentration over the range 0·025 to 0·5N (pH 12·4 to 13·3) had little effect on the diffusion current of the main peak. The concentration of 0·05N was finally chosen as most suitable for the assay of fermenter broth.

A peak corresponding to the second wave observed by Bricker and Vail (1951) was seen in all cases at about $E_{\frac{1}{2}} = -1{\cdot}65V$ (against S.C.E.). Its height increased with a decrease in lithium hydroxide concentration but did not affect the height of the main peak. The half-wave potential of the main diffusion peak was constant at about $-1{\cdot}57V$ (against S.C.E.) over the above range of lithium hydroxide concentration but was less negative at lower pH levels. A third peak was sometimes observed at about $E_{\frac{1}{2}} = -1{\cdot}3V$ (against S.C.E.) in less pure samples. The addition of lithium chloride, as advocated by Whitnack and Moshier (1944), Warshowsky and Elving (1946) and Elving (1948), for the polarographic determination of aldehydes, had no effect on the determination. Variations in temperature over the range 16·5 to 20° caused no change in diffusion current so that rigid temperature control was not required.

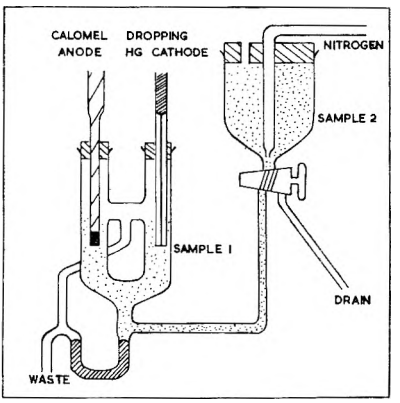

FIG. 1. Diagram of polarographic cell designed for semi-continuous working.

Oxygen was removed from the sample by purging with nitrogen. The diffusion current increased with the time of bubbling up to 5 min. but thereafter decreased steadily because of alkaline degradation of streptomycin. Strict time control was thus necessary between addition of the base electrolyte and the polarogram recording.

Assays were made at two dilution levels because under such conditions the method was more reliable and could be subjected to validity testing. The results on 80 (high) and 40 (low) units streptomycin/ml. were calculated by comparison with approved standard streptomycin, this material was identical with the current international standard of 780 units/mg. These levels were within the range of rectilinear streptomycin response and were convenient for the polarograph scale. Such concentrations also allowed sufficient dilution for the assay of fermenter broth as described below. When these concentrations were diluted with an equal volume of 0·1N lithium hydroxide they gave diffusion currents of approximately 0·1 and 0·05 μA respectively.

The method was particularly convenient when used with a cell as shown in Fig. 1. This cell permitted simultaneous nitrogen purging and

polarogram recording. It also prevented movement of the mercury dropper during any series of determinations. The volume of sample for purging was about 40 ml. whereas that of the electrode compartment was about 4 ml. This ten to one ratio of volumes ensured proper emptying of the contents of the cell compartment and replacement by successive nitrogen purged sample. There was also facility for rinsing the purging compartment.

Assay of Fermenter Broth

Weighed samples of fermenter broth were adjusted to pH 2·0 with N sulphuric acid, diluted five times with water, allowed to stand for about 30 min. at room temperature and filtered. The filtrate was then adjusted to pH 7·0 with N sodium hydroxide and diluted to high and low levels of 80 and 40 units/ml., respectively. An equal volume of 0·1N lithium hydroxide was added to each solution: these were immediately purged with nitrogen for 5 min. and the polarogram then recorded. The diffusion current wave height from fermenter broth, unlike that from solutions of pure material, varied with the concentration of the base electrolyte over the range 0·025 to 0·5N. The diffusion current however approached a flat maximum with 0·05N lithium hydroxide and showed the minimum of variation with small fluctuations about this value. The observed decrease in diffusion current with increase in lithium hydroxide concentration is thought to be due to the relatively high rate of alkaline degradation of streptomycin in fermenter broth. Sample dilution has also been found to affect the assay probably by diluting out the effect of interfering substances which act as suppressors. Dilutions of five and ten times gave results within about 30 and 10 per cent respectively of those by the microbiological method (Brownlee and others, 1948), whereas dilutions of twenty times or greater gave results generally within 5 per cent. A dilution of at least twenty was thus a necessary feature of the method. There appeared to be little difference in results between an internal or an external standard. The latter was adopted as it was more convenient. It had a sample : standard concentration ratio closer to unity and was more suited to validity testing. A series of twenty broths was assayed by the polarographic and microbiological methods and the results were compared statistically. The average difference between individual results was less than 5 per cent and the means showed no significant ($P = 0{\cdot}10$) difference in a Students '*t*' test. Both methods thus gave similar results. The standard deviation between replicates was about $\pm 2{\cdot}5$ per cent which was generally better than the corresponding error in the microbiological method.

Assay of Process Recovery Samples

The process recovery samples were mainly dilute sulphuric acid eluates from columns of Amberlite IRC-50 resin and spent fermenter broths. Resin column eluates were assayed by the procedure used for pure samples and the results were in good agreement with those by microbiological assay.

Spent broths were difficult to assay because of the low concentration of streptomycin and the high proportion of impurities. The difficulties were overcome, however, by re-adsorption and elution of the sample on a relatively large Amberlite IRC-50 column (100 ml. sample and 20 ml. resin). This treatment effected sufficient purification and gave results which were within 10 per cent of those by microbiological assay; this was substantially better agreement than obtained with the maltol method.

Hydrogenation of Streptomycin Concentrate

The presence of 1 per cent w/w streptomycin as an impurity in dihydrostreptomycin is readily detected by the polarographic method, which is more sensitive than the maltol method usually adopted. The examination of two hydrogenation experiments by both methods is shown in Table I. The results by polarography suggest that in both instances the residual streptomycin is less than 0·5 per cent w/w whilst by the maltol method a figure of about 1 per cent is given.

TABLE I

HYDROGENATION OF STREPTOMYCIN CONCENTRATE AS FOLLOWED BY POLAROGRAPHIC AND MALTOL ASSAY

Run No.	Hydrogenation time, hr.	Polarographic assay		Maltol assay per cent of original strep.
		Units/ml.	Per cent of original strep.	
1	Nil	188,800	100·0	100·0
	7·5	15,000	7·9	9·2
	11·5	1,300	0·7	1·5
	12·3	810	0·4	1·0
	13·0	586	0·3	0·9
2	Nil	173,000	100·0	100·0
	6·0	26,400	15·0	20·0
	12·0	2,650	1·5	3·2
	16·0	840	0·5	1·5
	18·0	545	0·3	1·1

DISCUSSION

The polarographic method using a derivative circuit for the assay of streptomycin compares favourably with other chemical methods. The maltol method in particular is subject to interference from streptomycin-like compounds which respond as streptomycin. Such impurities are apparently polarographically reduced at a half-wave potential which differs from streptomycin; they are thus excluded from the result. Derivative polarography is usually carried out at the expense of sensitivity, but the improved resolution obtained in this case enables a more satisfactory use of the high sensitivity ranges of the instrument. The sensitivity of the polarographic assay is similar to that of the microbiological method.

Acknowledgements. We are indebted to Mr. R. M. Roberts for designing and constructing the polarograph cell shown in Fig. 1, and to Miss S. Southern and Mr. K. Galloway for technical assistance.

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The paper was presented by MR. GOODEY.

STUDIES ON THE POSTIRRADIATION OXYGEN EFFECT IN BACTERIAL SPORES

BY A. TALLENTIRE AND N. A. DICKINSON

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Received May 21, 1962

RECENT work involving exposure of dried bacterial spores to ionising radiations shows that the presence of oxygen after irradiation increases lethal damage caused by energy absorption (Powers, Webb and Kaleta, 1960; Tallentire and Davies, 1961). It is of particular interest in the application of "radiation sterilisation" that the lethal efficiency of a dose of radiation can depend on the storage conditions of the irradiated material. Preliminary experiments described are designed to elucidate the mechanisms of these postirradiation effects.

Kaolin powder contaminated with spores of *Bacilius subtilis* (NCTC 3610) (Tallentire and Davies, 1961) was used for the experiments. Weighed samples were dried at less than 10^{-5} mm. of mercury for 6 hr., refrigerated traps being incorporated in the drying train. Samples were then sealed under vacuum and treated at 22° with different doses of gamma-radiation from a cobalt-60 source. After irradiation, samples were stored in controlled gaseous atmospheres at 25°. Control samples were treated in an identical manner omitting only the irradiation step. The criterion of lethal damage chosen was the inability of the spore to give rise to a colony on incubation in nutrient agar. From colony counts of samples yielding surviving fractions less than 0·5, exponential dose/survival curves were constructed and the slopes of these estimated using the expression

$$\text{Surviving fraction} = e^{-kD}$$

where k is the slope and D the dose in Krad. The slope is used as a measure of the lethal efficiency of the radiation, higher values of k indicating greater efficiency.

The highest level of lethal efficiency shown in Fig. 1 is that resulting from postirradiation storage of spores in oxygen for 48 hr. (k = 0·045 $Krad^{-1}$). The lowest efficiency is that for identical oxygen treatment preceded by exposure to nitric oxide for 15 min. (k = 0·010 $Krad^{-1}$). This prevention of the postirradiation oxygen effect by nitric oxide confirms the previous work of Powers, Webb and Kaleta (1960) who concluded that such treatment with nitric oxide removes radiation-induced free radicals which on exposure to oxygen combine to produce damage lethal to the spore.

An intermediate value of k = 0·023 $Krad^{-1}$ results from exposure to oxygen for 30 min., and the same slope is obtained when spores are stored in oxygen for 30 min. then in a vacuum of less than 10^{-5} mm. of mercury for 47·5 hr. When the oxygen pressure is restored to 760 mm.

so that the total storage period in oxygen is 48 hr., k is increased to 0·046 Krad^{-1}, a value almost identical with that obtained from uninterrupted postirradiation exposure to oxygen for 48 hr. (0·045 Krad^{-1}, see Fig. 1). Clearly, the development of the postirradiation oxygen effect can be arrested by removing the oxygen and can be restarted by re-admitting oxygen to the dried spore system. Treatment with nitric oxide between exposures to oxygen, however, prevents further development of the oxygen effect. Thus exposure of irradiated spores in turn to oxygen for 30 min., to nitric oxide for 15 min., and then to oxygen up to 48 hr. gives a value for k of only 0·026 Krad^{-1}.

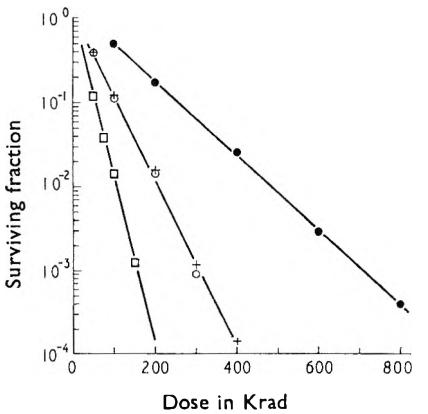

FIG. 1. Gamma irradiation of secondary dried *B. subtilis* spores in vacuum with different postirradiation storage treatments at 25°.

□——□ 760 mm. O_2 for 48 hr. Slope k = 0·045
\+——+ 760 mm. O_2 for 30 min. Slope k = 0·23
○——○ 760 mm. O_2 for 30 min. and then vacuum (less than 10^{-5} mm. of mercury) up to 48 hr. Slope k = 0·23
●·——● 90 mm. NO for 15 min. and then 760 mm. O_2 up to 48 hr. Slope k = 0·10

From these preliminary results and accepting the views of Powers, Webb and Kaleta (1960) on the scavenging role of nitric oxide, we infer that with samples dried as described above, the postirradiation oxygen effect in spores results from an association of free radicals with oxygen. In addition, we show that potentially harmful radicals can be removed even after exposure to oxygen and after partial development of the oxygen effect. Since these radicals are also harmless when the oxygen pressure is reduced, we propose that gaseous oxygen maintains the existence of an unstable oxygen-radical complex which itself is responsible for the postirradiation oxygen-dependent lethal effect.

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Discussion

The paper was presented by Dr. Tallentire. The following points were made in the discussion.

The response of spores to X-radiation was qualitatively and quantitatively the same as that from the cobalt 60 source. After irradiation all surviving spores germinated, but not all went through sufficient divisions to make a colony.

Short Communication

THE EFFECT OF AGE ON THE VIABILITY OF *PENICILLIUM NOTATUM* SPORES IN WATER AND SOLUTIONS OF PHENOL

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Immature spores have a slower rate of germination and less of them germinate than mature spores; they are also more sensitive to unfavourable temperatures. Physiologically senescent spores behave similarly (Cochrane, 1958). We report a variation in resistance to phenol of *Penicillium notatum* spores of different ages.

Experimental

Oxoid Sabouraud glucose agar slopes were inoculated from a stock culture of *P. notatum*, incubated at 28° and used at intervals from the third to the twenty-eighth day to prepare spore suspensions. The viability of the spores in water and after exposure for 10 and 20 min. to a 1 per cent solution of phenol and for 10 min. to a 1·125 per cent solution was determined at 25°. The methods of preparation of spore suspensions and evaluation of fungicidal activity were those of Chauhan and Walters (1961; 1962).

Results and Discussion

Table I shows that maximum resistance to both solutions of phenol is found in spores from 5–10 day cultures. Table II shows that when stored in water at 4° the resistance to phenol of an aqueous suspension of a 10-day old culture remained unaffected for seven days. Moreover, the subsequent decrease in resistance to phenol is less marked than when the spores were allowed to age in culture (cf. Tables I and II).

TABLE I

Effect of age on the viability of *P. notatum* spores in water and solutions of phenol

Phenol concentration (per cent)	Contact time (min.)	Per cent germination: Spores from culture of age (days)								
		3	5	8	10	12	15	19	24	28
0	20	99	97	97	96	97	94	93	92	91
1·0	10	20	70	66	66	56	42	22	23	25
1·0	20	13	56	55	55	37	22	15	14	9
1·125	10	5	19	21	20	12	5	2	<1	<1

As spores taken from a 3-day culture will not all have been formed at the same time, the oldest having an age of about 48 hr., some, although detachable and capable of germination, may not be fully developed, and

because of this might be expected to show less resistance to phenol than mature spores.

Maximum resistance to phenol is possessed by spores from 5–10 day cultures, which are presumably mature. When older than 10 days their resistance to phenol decreases with increasing age.

TABLE II

EFFECT OF STORAGE OF *P. notatum* SPORES* IN WATER AT 4° ON THEIR VIABILITY IN SOLUTIONS OF PHENOL

Phenol concentration (per cent)	Contact time (min.)	Per cent germination								
		Storage period (days)								
		0	3	7	11	15	25	42	120	320
0	—	97	96	94	97	95	96	93	96	92
1·0	10	65	66	63	57	54	57	49	28	6
1·0	20	53	52	56	45	43	44	27	18	1
1·125	10	25	27	22	18	18	19	12	6	0

* From a 10-day old culture.

For the evaluation of fungicides, spores possessing maximum resistance to the test fungicide should be used. With phenol and *P. notatum* and the experimental conditions described, spores from 5 to 10-day old cultures which may be stored in water at 4° for up to 7 days, are suitable.

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The paper was presented by DR. WALTERS.