BRITISH PHARMACEUTICAL CONFERENCE

NINETY-EIGHTH ANNUAL MEETING, PORTSMOUTH, 1961

REPORT OF PROCEEDINGS

OFFICERS:

President:

H. S. GRAINGER, F.P.S., London

Chairman:

D. C. GARRATT, D.Sc., Ph.D., F.R.I.C., Nottingham

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.

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*).

*(Miss) M. A. BURR, M.P.S., Nottingham.
K. R. CAPPER, Ph.D., B.Pharm., F.P.S., D.I.C., London.
*T. REID, M.P.S., Haslemere.
G. F. SOMERS, B.Sc., Ph.D., F.P.S., M.I.Biol., Liverpool.
*H. STEINMAN, M.P.S., Manchester.
J. B. STENLAKE, D.Sc., Ph.D., F.P.S., F.R.I.C., Glasgow.
G. SYKES, M.Sc., F.R.I.C., Nottingham.
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

PORTSMOUTH, 1961

THE OPENING SESSION

The opening session of the Conference was held in the theatre of the South Parade Pier, Southsea, on Monday, September 18, with Mr. H. S. Grainger, President of the Conference (President of the Pharmaceutical Society), in the Chair. On the platform were the Chairman of the Conference (Dr. D. C. Garratt), the Lord Mayor of Portsmouth (Councillor R. Bonner-Pink), the Chairman and Secretary of the Local Committee (Mr. J. C. Bloomfield and Mr. N. L. Banks), the Honorary General Secretaries together with members of the Conference Executive Committee.

The President introduced the Lord Mayor of Portsmouth, 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 (Dr. D. C. Garratt), who delivered his address entitled "Analysis Analysed," which is printed in the *Journal of Pharmacy and Pharmacology*, 1961, **13**, *Suppl.* 9*T*–22*T*.

On the proposition of Mr. S. G. Stevens, seconded by Mr. T. C. Denston, the Conference accorded a vote of thanks to the Chairman for his address.

CIVIC RECEPTION

On the evening of Monday, September 18, a Civic Reception was given at the Guildhall. The guests were received by the Lord Mayor (Councillor A. Bonner-Pink) and the Chairman of the Local Committee (Mr. J. C. Bloomfield) and Mrs. Bloomfield. A dance was held after the reception.

THE SCIENCE SESSIONS

Meetings were held on Monday, Tuesday and Friday, September 18, 19 and 22 at the theatre of the South Parade Pier, or the Guildhall, the Chairman presiding. During the sessions the following 25 papers were communicated:—

1. The Stability of Solutions of the International Pyrogen Reference Preparation. By C. H. R. Palmer, B. Pharm. M.P.S. and T. D. Whittet, B.Sc., Ph.D., F.P.S., F.R.I.C.
2. The Removal of Air during Autoclave Sterilisation of Fabrics using Low Pressure Steam. By G. R. Wilkinson, F.P.S. and F. G. Peacock.
3. Improvement of Heating of Bottled Fluids during Autoclave Sterilisation using Low Pressure Steam. By G. R. Wilkinson, F.P.S. and F. G. Peacock.
4. A Slope-ratio Design for Accelerated Storage Tests. By J. P. R. Toothill, B.Sc., A.R.I.C.
5. Ethylene Oxide Sterilisation—Some Experiences and Some Practical Limitations. By A. Royce, F.P.S. and C. Bowler.
6. The Preparation and *in vitro* Evaluation of Some Modified Aluminium Hydroxides as Gastric Antacids. By B. K. Davison, B.Sc., Ph.D., F.R.I.C. and R. E. Schaffer, B.Sc., Ph.D.
7. Neuromuscular Blocking Agents. Part IX. Some Short-Acting *NNN*-Trisonium Esters. By Fiona MacLeod Cary, B.Sc., J. J. Lewis, M.Sc., F.P.S., J. B. Stenlake, D.Sc., Ph.D., F.P.S., F.R.I.C. and W. D. Williams, B.Pharm., Ph.D., F.P.S., A.R.I.C.
8. The Anti-inflammatory and Antidiuretic Actions of Fractions Obtained from Xanthoglabrol. By R. Best, B.Pharm. and R. S. H. Finney, B.Sc., M.Sc.
9. The Determination of Hexachlorophane and other Phenols in Pharmaceutical Preparations by a Δ E Spectrophotometric Method. By D. A. Elvidge, B.Sc., A.R.I.C. and B. Peutrell, B.Sc.
10. The Spectrophotometric Determination of Thalidomide in Body Fluids. By J. N. Green, B.Sc., Ph.D. and B. C. Benson.
11. The effect of a Sulphated Polysaccharide upon the Diffusion of Pepsin through Mucin. By W. Anderson, B.Sc., Ph.D., F.P.S.
12. Ion-exchange Chromatography on Alginic Acid of Certain B-Group Vitamins. By J. S. Foster, B.Sc. and J. W. Murfin, B.Sc., F.R.I.C.
13. On the Standardisation of Thyroid B.P. By C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C. and K. L. Smith, M.P.S.

14. Comparison of Biological and Chemical Assay of Thyroid. By F. W. Webb, B.Sc.
15. The Strength of Compressed Tablets. III. The Relation of Particle Size, Bonding and Capping in Tablets of Sodium Chloride, Aspirin and Hexamine. By E. Shotton, B.Sc., Ph.D., F.P.S., F.R.I.C. and D. Ganderton, B.Pharm., M.P.S.
16. A New Method for the Determination of Barbiturates. By I. M. Roushdi, B.Pharm., Ph.D., H. Abdine, B.Pharm., Ph.D. and A. Ayad, M.Pharm.
17. The Flask Combustion Technique in Pharmaceutical Analysis: Mercury-containing Substances. By C. Vickers, B.Sc., A.R.I.C. and J. V. Wilkinson, B.Sc.
18. 4-Alkoxypiperidines Related to Reversed Esters of Pethidine. By A. F. Casy, B.Sc., Ph.D., F.P.S., F.R.I.C. and A. H. Beckett, D.Sc., Ph.D., F.P.S., F.R.I.C.
19. A Specific Solvent-extraction Method for the Determination of Trace Quantities of Fluoride. By C. A. Johnson, B.Sc., B.Pharm., F.P.S., F.R.I.C. and M. A. Leonard, B.Sc., Ph.D.
20. Entrainment and Flooding in Vertical Still-heads. By A. J. Evans, B.Pharm., Ph.D., E. Shotton, B.Sc., Ph.D., F.P.S., F.R.I.C. and D. Train, M.C., B.Pharm., B.Sc.(Eng.), Ph.D., F.P.S., F.R.I.C., M.I.Chem.E.
21. Studies on Bacterial Populations in Solutions of Phenols. Part II. The Influence of Cell-exudate upon the Shape of the Survivor-time Curve. By H. S. Bean, B.Pharm., Ph.D., F.P.S. and V. Walters, B.Pharm., Ph.D., F.P.S.
22. The Assay of Enzyme Activity by the Plate-diffusion Technique. By D. V. Carter, B.Sc. and G. Sykes, M.Sc., F.R.I.C.
23. The Influence of the Variations in Solubilising Properties of Polysorbate 80 on the Vitamin A Palmitate: Polysorbate 80: Glycerol: Water System. By P. F. G. Boon, B.Sc., A.R.I.C., C. L. J. Coles, B.Pharm., F.P.S. and M. Tait, B.Pharm., M.P.S.
24. Phase Equilibria in Systems Containing Commercial Non-ionic Surface-active Agents and Benzene. By B. A. Mulley, B.Pharm., Ph.D., A.R.I.C.
25. Some Observations on the Flow Properties of Barium Sulphate Suspensions. By J. E. Carless, M.Sc., B.Pharm., Ph.D., F.P.S. and N. C. Chenoy, B.Pharm.

THE CONFERENCE LECTURE

A lecture on "Interferon" was given on Wednesday, September 20, by Dr. A. Issacs. The Chairman presided. The lecture is printed in the *Journal of Pharmacy and Pharmacology*, 1961, **13**, *Suppl.* 57*T*–61*T*.

THE SYMPOSIUM SESSION

A symposium on "Skin Medication" was held on Thursday, September 21. The Chairman presided. The introductory papers were by Drs. F. J. Ebling, A. Jarrett and Mr. J. W. Hadgraft. The meeting is reported in the *Journal of Pharmacy and Pharmacology*, 1961, **13**, *Suppl.* 25*T*–56*T*.

PROFESSIONAL SESSIONS

With the President of the Conference, Mr. H. S. Grainger, in the Chair, professional sessions were held on the mornings of Tuesday, September 19, when Mr. H. E. Chapman read an introductory paper to the subject "Impact of Restrictive Practices Legislation on Pharmacy and the Pharmaceutical Industry," and Friday, September 22, when Mr. F. W. Adams introduced the Report of the Committee on the General Practice of Pharmacy. Full reports of the papers and discussions were published in the *Pharmaceutical Journal*, 1961, **187**, 265–271; 291–306; 390–393.

THE CLOSING SESSION

The closing session of the Conference was held on Friday, September 22, in the theatre of the South Pier, the Chairman presiding.

Vote of Thanks to Local Committee

The Chairman called on Mr. E. Knott to propose a vote of thanks to the Local Committee. This was seconded by Mr. M. H. Thomas. Mr. J. C. Bloomfield

(Chairman of the Local Committee) replied to the vote of thanks. The Chairman then presented to the Portsmouth and District Branch an inscribed gavel provided by the Bell and Hills Fund. Mr. N. Francis (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-eighth 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 sixty-eight members elected by the Executive who are not members of these Societies.

Reports on 1960 Meeting.—The report of the meeting of the Conference at Newcastle upon Tyne, 1960, together with the science and symposium papers and discussions were published as a supplement to the twelfth volume of the *Journal of Pharmacy and Pharmacology*. Thd papers and discussions at the Professional Sessions were published in the *Pharmaceutical Journal*, Series IV, Volume 13[illegible].

Conference Papers, 1961.—Thirty-three research papers were submitted before the closing date. Nineteen full papers and six 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, 1961.—An innovation at the 1961 Conference at Portsmouth was the inclusion in the programme of a Conference Lecture by a distinguished invited speaker, on this occasion Dr. A. Isaacs of the National Institute for Medical Research, on "Interferon." 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 (Dr. D. C. Garratt), the immediate Past-Chairman (Professor W. H. Linnell) and the Senior Honorary General Secretary.

In view of the decision of the Council of the Pharmaceutical Society to increase the price of the *Journal of Pharmacy and Pharmacology* to members of the Pharmaceutical Society, the subscription of elected members of the British Pharmaceutical Conference as from January 1, 1962, will be £2 15s. 0d.

Future Meetings.—An invitation will be presented at this meeting for the Conference to meet in Liverpool during the week commencing September 3, 1962. The Centenary Meeting of the Conference will be held in London during the week commencing September 2, 1963. Your Executive has provisionally accepted an invitation to hold the Conference in Edinburgh in 1964 during the week commencing September 14. 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.—Your Executive has appointed a Sub-Committee to review the Constitution of the Executive and the Rules of the Conference. Any proposals will be reported at a subsequent meeting of the Conference.

Officers and Executive of the Conference.—Your Executive has nominated the following Officers and Members of the Executive for 1961–62:

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

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 one other, 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 1961–1962.

ACKNOWLEDGEMENTS.—The Executive wishes to record thanks to the Chairman, Officers and Members of the Portsmouth 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. D. W. Hudson proposed the acceptance of the report and the election of officers of the Conference for the ensuing year. Mr. D. A. Norton seconded.

Mr. J. C. Hanbury thanked the Conference on behalf of the newly-elected officers.

TREASURER'S REPORT

Mr. H. G. Rolfe presented and proposed the adoption of the following Report and Statement of Accounts for the year 1961:

In relation to the audited accounts for the year ended December 31, 1960, it should be mentioned that the Post Office Savings Bank Account was closed during that year.

The Local Committee Fund of £250 having been repaid by the Newcastle upon Tyne Local Committee, was loaned to the Portsmouth Local Committee in respect of the 1961 Conference.

The subscriptions of the elected members, including a composition fee from the Pharmaceutical Society of Northern Ireland, amounted to £142 5*s.* 0*d.* 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, 1960

Expenditure	£	*s.*	d.	*Income*	£	*s.*	*d.*
Gavel—memento to Newcastle and District Branch	8	17	6	Interest on 2½% Consols	40	5	0
Replica of Chairman's Badge, engraving, etc.	7	17	0	Interest on 3% Savings Bonds	6	0	0
Engraving Edmund White Golf Trophy		10	0	Interest on 3% Exchequer Stock	15	0	0
Ribbon for Chairman's badge		15	6	Interest on P.O. Savings Bank Account		1	3
Expenses of Speakers	25	0	6	Interest on Bank Deposit Account	3	11	2
Income Tax	20	15	0	Donation from Pharmaceutical Society of Northern Ireland	25	0	0
Surplus carried to Accumulated Fund	51	1	11	Donation from Pharmaceutical Society of Ireland	25	0	0
	£114	17	5		£114	17	5

BALANCE SHEET AT 31 DECEMBER, 1960

Liabilities	£	*s.*	*d.*	*Assets*	£	*s.*	*d.*
Accumulated Fund, as at 31.12.59:	2,008	15	2	Investments at cost (*a*) £1,610 2½% Consols (Donation by the late Alderman Clayton of Birmingham)	1,250	0	0
Add: Surplus 1960	51	1	11	(*b*) £200 3% Savings Bonds 1960–70	200	0	0
	2,059	16	1	(*c*) £500 3% Exchequer Stock 1962–63 (Total Market value at December 31, 1960: £1,343)	473	4	10
Local Committee Fund: Donation from London Committee, 1953	250	0	0		1,923	4	10
				Stock of Replicas (5) of Chairman's Badge	36	15	0
				Loan to Portsmouth Local Committee	250	0	0
				Cash at Bank—			
				Current Account	13	12	11
				Deposit Account	86	4	4
	£2,309	17	1		£2,309	17	1

Audited and found correct
J. C. HANBURY
T. HESELTINE
May 17, 1961

The President seconded, and the Report was adopted.

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.
1986	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.

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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 1962

Mr. H. Humphreys Jones on behalf of the Liverpool and District Branch, extended an invitation to hold the Conference in Liverpool in 1962.

Mrs. I. M. Z. Elliott proposed that the invitation be accepted, and Dr. W. Mitchell seconded. The vote was put to the meeting and unanimously carried.

VOTE OF THANKS TO CHAIRMAN

Mr. D. Stephenson proposed a vote of thanks to the Chairman.

Dr. J. E. Carless seconded. The vote was put to the meeting by the President and carried with acclamation.

Dr. Garratt 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.

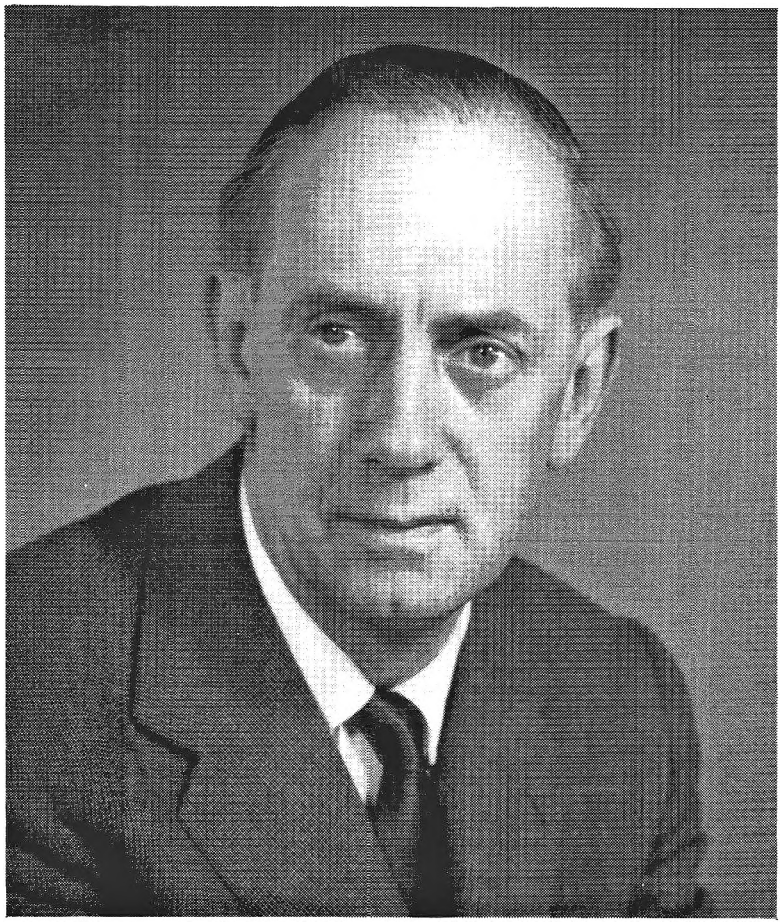
4. The Officers of the Conference shall be selected at each annual meeting by those present. Nominations for membership of the Executive Committee may be made at the annual meeting by the outgoing Executive and shall include nominations which have been received by the Honorary Secretaries in writing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



D. C. GARRATT

Chairman, 1961

BRITISH PHARMACEUTICAL CONFERENCE
Portsmouth, 1961

Chairman: D. C. Garratt

CHAIRMAN'S ADDRESS

ANALYSIS ANALYSED

A More Rational Approach to Pharmaceutical Control

Few would dispute that in the last decade analytical chemistry has developed its potential value more rapidly than in the previous fifty years.

Analysis has now broken out from its earlier restricted sphere and become a completely new concept with the right to be considered a discipline in itself. Its phenomenal expansion, mainly due to new instrumental methods and a widening of their application, has been as much of value in the field of pharmaceutical production as in any other branch of industry.

This increase in the availability of analytical tools and techniques coincides with the necessity for more rigorous control of the potent drugs now available. Stricter control is also necessitated by modern methods of manufacture and filling, among which might be mentioned the use of new materials as containers, plastic closures, aerosol packs, and more exacting requirements in packaging and storage for overseas markets, and this further increases the responsibilities of the pharmaceutical analyst. With this change in the scope of analysis it is inevitable that more elaborate testing is applied.

The trend in the stringency of drug control can be seen by comparison of successive editions of the B.P. or B.P.C. Unfortunately this results in greatly increased costs of analysis; the instruments are costly and the skilled analyst has become more specialised and thus more expensive. This increase in costs, although small compared with other factors—particularly the price of the drug itself—must nevertheless be reflected in the price of the finished preparation. Further to complicate this situation legislation is contemplated for the stricter control of the price of medicinal products.

The rising cost of the National Health Service and, of course, the normal commercial hope of more economical production of drugs should justify a search for all causes of the present costs. The cost of analytical control must be considered amongst these and not accepted without question—which it often is—as a necessary overhead. As a contributory factor in the general cost of drugs, it would be as well to find its value and make some attempt to reduce it. It is my intention now to consider in more detail some of the reasons for this increasing "overhead", to outline some ideas on methods for reducing it and to question whether we are making the best use of our analytical resources. I shall digress from the main theme from time to time to consider various dependent

issues where it seems relevant to do so, but not losing sight of the main topic of cost. I shall conclude with some suggestions on the overall problem of the general aspects of pharmaceutical control.

A large analytical laboratory dealing with a multiplicity of products has the advantage that it can obtain information on costs which is of general application; very distorted figures can be obtained from assessment of more specialised work. The figure often calculated, particularly in the United States, is the percentage of the total production costs that is due to control. This may be unrealistic when the production costs include basic materials of substantial value and a better comparison is to relate control to the total overheads of production. To quote actual figures would be misleading since each firm has a different costing system but for my own laboratory, cost of analytical control as a percentage of total production overheads has risen significantly over a recent five year period from 7·07 to 8·02.

Further, a breakdown of these costs shows that staff salaries and pensions accounted for 74·1 per cent in the first year of this period and 79·1 per cent in the fifth year and it is fair to conclude that the major reason for increased costs is the increase in the market value of analysts, particularly if they are qualified. Therefore the most effective way to reduce costs is to reduce staff. Moreover, at the present time, analysts are at a premium and to reduce the need for them will help relieve the scarcity. It might be thought that a saving of costs could be effected by replacing qualified staff by skilled technicians of National or Higher National Certificate standard for much of the laboratory work where less responsibility is required, but at the present time technicians are even scarcer than graduates.

A Need for Analysts

One might pause here to enquire why there is a shortage of analysts. This is a national problem and not confined to one section of industry although, with the necessity for rigorous control in the rapidly expanding field of pharmaceutical products, the shortage is probably more acute there than elsewhere. The fault lies at the door of both industry and the academic institutions. In the past, the analyst has been the "poor relation" of the chemical profession and little encouragement had been given to any chemist to adopt analysis as a career. It must be admitted that much of the control work possible with the limited tools and methods available up to post-war times could have been done by a lower calibre of chemist than is needed today. Only if an analyst had a wide experience was he in demand and much of pharmaceutical analysis was unpublished work. However, since then the wide variety in methods available and the manipulative skill required in their application has increased so rapidly that it has become impossible for them to be assimilated by any but chemists devoted to analytical work as a profession; indeed specialists are already emerging from within the analytical field.

The rapidly increasing importance of the subject has been recognised in appointments to Chairs of Analytical Chemistry at Birmingham and

Belfast. Colleges of Advanced Technology in their contacts with Industry have realised the need for education in this specialised "profession within a profession" and are establishing Readerships in Analysis. Postgraduate courses are also being prepared. Nevertheless we must be deeply concerned not only at the scarcity of properly trained analysts but also at the apparent disinclination of chemists from the Universities to choose analytical chemistry as a career—mainly because they have little idea what modern analytical chemistry offers; serious active consideration should be given to means of inducing graduates to enter this kind of work.

No one would doubt that it is as necessary as in any other chemical career to give potential analytical chemists a good basic knowledge of chemistry in their degree course. However, I venture to suggest that analytical chemistry is becoming so important nationally that the time has come when more consideration should be given to the creation of an applied chemistry course in analytical chemistry, where the curriculum, at least in the final year, could be devoted to training in the specialised needs of the subject, with graduation in analytical chemistry. Analytical chemistry could also be accepted as a subject for diplomas in technology. Thus industry would be saved much valuable time in such training after graduation.

Pharmaceutical analysis is one specialised part of the analytical field with its own particular skills and it has undoubted justification for consideration as a branch of pharmaceutical study in its own right. The subject is of sufficient extent to be considered suitable for Diploma of Technology courses or, better, for a specialised course in pharmaceutical analysis after a Diploma of Technology. Pharmacy should not let pharmaceutical analysis remain largely in the hands of non-pharmacists; it is surely a strange situation that a large proportion of the leading pharmaceutical manufacturing houses in this country now employ a non-pharmacist as chief analyst.

Pitfalls of Assay Changes

The main cause for the increase in the amount of analytical control is the increased testing required on potent drugs and their formulated products, especially those used parenterally where it is necessary to do tests for sterility, toxicity, pyrogens and so forth. The most valuable contribution to the saving of man-power in pharmaceutical laboratories is the replacement of chemical methods by physical techniques and the adaptation of these to routine analysis. Papers have been given to this Conference from time to time on the application of such techniques as emission spectrography, flame photometry, ultra-violet, infra-red and fluorescence spectroscopy and gas-chromatography, all of which offer considerable advantages of speed in routine analysis.

It is certainly necessary to take all reasonable precautions against impurities in drugs but are all our tests necessary, each telling us something of value? If we are to spend money on analytical control let it be on testing that is worthwhile! Are all our expensive biological tests necessary? Need Protamine Zinc Insulin be tested for delayed activity if it contains

the right amount of insulin and protamine and the pH is correct? In replacing a "classical" determination by a modern technique are we gaining anything or just persuading ourselves that because it is new it must be better? Is it less expensive in apparatus, reagents, and time? Every time we change a specification or method of analysis we should be sure that it is an improvement.

A carefully controlled comparison was made of the cost of five different methods of assay available for determining the purity of one pharmaceutical chemical.

	Actual manipulative time (min.)	Total time	Cost of analyst's time s. d.	Reagent cost s. d.	Total cost s. d.
Non-aqueous titration	17	17 min.	8 6	4	8 10
Kjeldahl nitrogen	22	3½ hr.	11 0	7	11 7
Extraction of base	30	1½ hr.	15 0	3 6	18 6
Bromination	17	50 min.	8 6	6	9 0
Ultra-violet absorption assay (solvent, water)	20	20 min.	10 0	0	10 0

Since only costs are being considered, let it be assumed that all methods are of equal accuracy. Comparison of these figures indicate that from consideration of both cost and time extraction is least economical. The others are little different in cost but the Kjeldahl method takes a long total time and bromination rather long. Non-aqueous titration and ultra-violet absorption are equivalent and take least time; since ultra-violet absorption assay is the more specific it is the method of choice. Nevertheless, had the chemical needed a solvent other than water for solution the cost of this would have significantly affected the assessment. For example, had the solvent been cyclohexane, the reagent cost would have been 1s. 8d., with chloroform, 3s. 4d., and with alcohol, 6s. 0d.

The compilers of our official publications should bear these considerations in mind.

Assay Precision

In my opinion there is little justification for many of the assays at present applied to the large range of pure synthetic drugs, yet it would seem that the aim is to include an assay wherever possible. Much time must be spent in laboratories all over the country indulging in this extravagance. Before deciding on a minimum acceptable purity for production material, the assay must be fixed. In most cases no figures are available of the variation to be expected in replicate analyses and I an confident that, with few exceptions, this assessment is not done; without this knowledge the purity figure fixed as minimum is meaningless. Strange though it may seem a bioassayist has an advantage over his chemical counterpart since, although his errors are large he does at least know their extent. How can the tightening of a standard for a drug from 98·5 to 99·0 per cent be justified when assay differences of 0·5 per cent are accepted in duplicate tests? It is very comforting to get an assay

result within the minimum standard demanded; if it is not the assay is repeated; with a good result on this second test, of course the first one was an error and the material is "up to standard". Or the more careful analyst might do a third assay to decide which of the earlier two results was correct; it is an even chance that the third result will agree within a reasonable difference with one or the other. Again, if it is close to the upper figure the quality of the material is accepted as satisfactory although the conclusion can be far from the truth.

There are a number of assays based upon measurement of ultra-violet extinction. This is basically a bad technique to use unless reference standard materials of high purity are available for analysis by comparison. Up to now the E(1 per cent, 1 cm.) values chosen for calculation have been obtained from data supplied by manufacturers and users and are either an average from a recorded range, which may be quite wide, or the highest known value; therefore if the E(1 per cent, 1 cm.) value for the pure material is not known the standard adopted is based on a commercial product as optimum and the final minimum purity figure is correspondingly low. Coupled with this anomaly the existence of instrumental variation must be recognised. Hence the present use of ultra-violet spectrophotometric assay as a measure of the purity of a fine chemical is unrealistic.

An even more reprehensible setting of an assay standard is associated with an added tolerance allowed because of the known non-reproducibility of results by the assay method adopted. This may be acceptable when determining the amount of an ingredient incorporated in a formulation, but as a basis for assessing the purity limit for a chemical it can have no value since it is allowing an added impurity tolerance up to the spread of the assay precision.

Although I would prefer to see most assays of pure substances deleted since they are only an added identity test of little specificity and the criteria of purity are concerned with the other substances present, assays are likely to stay in the present form of monograph or specification. If so, they can only be made worthwhile, when the precision of the assay is unknown, if the sample under test is assayed against a standard material of known purity under identical conditions. In this way, for example, extinction measurements by differential spectrometry can be applied to a limiting precision (due to variables in the instrument, light source, light scatter, cell imperfections) of 0·1 per cent.

Hence there is an urgent need for a bureau for maintainance of a collection of highly purified medicinal chemicals in which the amounts of any impurities are accurately known. An international collection is so cumbersome a means of establishing standards that a national collection is the best workable method. It would take some time to collect the necessary materials but if, in the future, manufacturers introducing a new drug on the market were required to provide an adequate supply of pure material as primary standard the task would be lightened considerably. The bureau should be under the control of, or in collaboration with, an organisation where full facilities are available for accurate physical measurements and must be prepared to study the materials

collected for standards with the full armoury of modern techniques (X-ray crystallography, mass-spectrometry, zone refining, and so on) in assessing purity.

There is also a need for analytical research for more specific tests of identity and assay. The analyst can hardly be blamed for shortcomings in this matter since the multiplicity of drugs being introduced in such a short period has given him far too little time. However, it is a vital need, particularly with the marketing of chemicals of closely similar chemical composition but different pharmacological activity or action. At present these often have little in the way of tests by which they may be distinguished, particularly when in admixture or in formulated products; one might mention, for example, stilboestrol and dienoestrol, prednisone and hydrocortisone, adrenaline acid tartrate and noradrenaline acid tartrate. Such problems are undoubtedly exercising the minds of various standardising committees.

The following example will illustrate the need for vigilance in the standards set for drugs. The application of the triphenyltetrazolium chloride assay (which is specific among steroids for 17,21-dihydroxy-20-oxosteroid) to different corticosteroids obtained from various sources, has shown that specimens can comply with all the official requirements but be deficient in content of 17,21-dihydroxy-20-oxosteroid to a considerable extent. This is illustrated in the comparison of analytical figures specified by the B.P. and U.S.P. monographs with those found on a sample of hydrocortisone acetate.

	B.P. standard	U.S.P. standard	Sample figures
Specific rotation	+157° to +167°	+158° to +165°	+159·1°
Loss on drying (maximum)	1 per cent	1 per cent	0·02 per cent
Sulphated ash (maximum)	0·1 per cent	negligible	0·02 per cent
Melting point	about 220°	216° to 222°	218·4°
Assay (ultra-violet)	96 to 104 per cent on dried material		101·3 per cent
"Tetrazolium" assay			90·5 per cent
Related foreign steroids		complies	

The sample would have satisfied a routine identity test by infra-red examination. This example does emphasise the need for devising tests of greater specificity. Paper chromatography will certainly have to play a part in characterising substances and detecting admixture.

Hence, there is a rapidly growing need for reconsideration of our conventional monographs and development of other methods of differentiation of organic substances, together with better appreciation of the true value of the purity tests used to establish the nature and quantity of impurities present to any significant extent. To implement this concept it must be presupposed that the latest instrumental techniques are now within the scope of the normally equipped laboratory; the plea that equipment is too expensive is no longer tenable since, as already indicated, the analyst is by far the greatest cost. Mention might be made of the greater significance of possible trace residues of highly toxic catalysts than of the so-commonly sought impurities, lead and arsenic. I suggest that

a monograph of the not-too-far-distant Pharmacopoeia might include such requirements as:

> Concentrate the impurities by the zone refining procedure (see Appendix), with a downward movement of the zones, at a rate of 2 cm. per hour, and 15 zone passes (carrying out the operation under nitrogen and maintaining the apparatus at a temperature of 0° C.). Remove sections 5 cm. long from the bottom, from the top, and from the centre of the column and prepare a mass spectrogram from each section (see Appendix).
>
> The section taken from the top of the column should show no more impurity than that taken from the centre of the column. Compare the mass spectrogram of the material from the bottom of the column with that from the centre of the column and estimate the amount of impurity in the bottom section. The amount of impurity indicated in the original material by this procedure should not be greater than 0·5 per cent.

A discussion on assay precision cannot be confined to the drugs themselves. Some latitude in the results can be tolerated when methods are applied to the determination of the quantity of drugs in formulated products since the present official limits are based on an allowance for manufacturing variation plus the minimum allowable purity of the drug used. Although it has already been shown that the standard for the drug used may be fallacious, the allowance for manufacturing error is generally more generous than a good commercial manufacturing firm would need (being given to cover extemporaneous preparation in pharmacies) so no difficulty is encountered in meeting the requirements. However, the more complex a formula becomes, the greater the errors of assay introduced and, therefore, even the present tolerances, generous though they may seem, may not be sufficient. Let me illustrate this with an example:

Compound Codeine Tablets were manufactured without the codeine phosphate; these tablets were powdered and intimately mixed with previously assayed codeine phosphate to give a known content of this ingredient within the B.P. limits. Portions of the powder were sent to analysts whose reputation for accurate work is beyond question with a request for a B.P. assay of codeine to be done; no indication was given that this was a collaborative trial. All results were within the B.P. limits but the spread was 9·2 per cent.

The result is not surprising; the assay is difficult, involving the determination of a small quantity of codeine in the presence of a large amount of other drugs, and many attempts have been made to improve its accuracy. The above example was only one of a number of collaborative trials with similar findings.

There have been, from time to time, allegations that certain less reputable manufacturing firms are deliberately using a quantity of drug to the minimum tolerance. This may be true but with such a spread of results possible are we sure we are justified in our contention, remembering that many firms may be showing higher results only because they add a small overage for safety and the particular assay may give low results generally?

Strict control of pharmaceutical manufacture is a necessity but more common sense must be shown in the judgment of borderline cases until more work has been done in assessing the precision of the assays (for which a considerable amount of investigation would be demanded) and

until national reference standard drugs are available for differential analysis. This thought is disturbing but analysts must be shaken out of their present apparent complacency regarding the accuracy of their results, since accuracy is essential to their professional reputation.

Hidden Costs

In assessing analytical costs the amount of testing of materials bought and manufactured and the cost of staff are obvious concerns, but there are smaller and hidden expenses which are worth mentioning; these added together are not to be ignored in the increasing costs of pharmaceutical control.

The need for analytical research into the precision of assays and for devising methods of greater specificity has been stressed. In this matter a re-assessment of older methods is worthy of consideration. There is also constant adaptation of new techniques to the special problems associated with pharmaceutical analysis. The comment is often made that such methods, usually involving expensive apparatus, are suitable for large laboratories but not applicable in smaller units. Admittedly larger laboratories have the facilities for the initial investigation and adaptation but many smaller laboratories either make no effort to apply these time-saving techniques or do not use their instruments fully, only applying instrumental techniques in official or recognised methods.

Industrial pharmaceutical laboratories do extensive voluntary work into analytical methods on behalf of official standardising committees and I know the committees are grateful for the help. Nevertheless the cost must be noted.

Control of packaging materials and packed goods and shelf-life assessment for all the new closures and presentations take up time not always directly assessed as analytical charge.

Another charge to the analytical services is sometimes overlooked. Pharmaceutical firms have to prepare a considerable number of documents to register their products overseas. Usually the importing country demands full specifications and methods of determination of all active ingredients—in some instances all ingredients, active or not.

A hidden cost of analysis may result from the time samples are held before reporting. For expensive drugs the value of the stock investment may be considerable.

The employment of trained method-study personnel—necessarily with a technical, and preferably analytical, background—is a subject worthy of attention in the attempt to reduce costs. The ancilliary services to a laboratory certainly justify investigation. Thus sampling may be too thorough; time and motion study methods may cut unnecessary travel between works and laboratory; a tidier and more efficient docket system may be possible. These and many other operations all bear investigation but before they are examined it is as well to give senior laboratory personnel a short course in method-study appreciation so that the incoming investigator meets with a tolerant reception and not an attitude of resistance to what might look to be criticism of the laboratory organisation.

The assessment of the analytical work itself by normal time and motion study techniques is more difficult and a critical evaluation of the activities of a large group of analysts dealing with an extensive and varied range of materials would be very time-consuming. The technique of "activity sampling" has much to recommend it. A method-study worker, using this technique, can obtain information on selected personnel in a comparatively short time and with a good assessment of the accuracy of his results, with the decided advantage of not upsetting the normal routine of those being studied. In this way one worker has been able to show in a matter of days that the paper work activities of a group of nine section heads occupy 26 per cent $\pm$ 4 per cent of their time and that about 73 per cent of this activity is devoted to one particular type of paper work. Such information enables one to assess how much method-study time might profitably be devoted to reducing paper work and which particular aspect of the problem to tackle first.

How is the value of method-study to be judged? Not necessarily—and certainly not only—by the reduction in the number of laboratory personnel or in other laboratory costs. It may be much more of a gain to be able to give a more efficient service at the same cost and with the same personnel, particularly if this were to be reflected in a reduction in the time taken for a sample to pass through the laboratory. However, sometimes a direct saving can be shown as, for example, in reducing by various means the usage of expensive solvents.

The very fact that method-study has been introduced and is being used will often stimulate laboratory personnel to examine closely the procedures in which they are involved and to make useful modifications without the direct intervention of method-study personnel. Some of the most valuable and, certainly, most acceptable schemes arise in this way.

It should be every analyst's concern to consider what can be done to reduce costs or at least to discourage their increasing. It has already been stressed that drug-standardising committees need to consider the comparative expense, particularly regarding the time required, for tests otherwise of equal merit and to re-assess the necessity for the present tests. Industrial pharmaceutical laboratories should also do this for reasons of internal economy, remembering that the B.P. and B.P.C. permit alternatives to the methods of determination described provided these are of equivalent accuracy. Working time can be considerably reduced by the use of such techniques as titration with EDTA to replace gravimetric methods, the use of paper chromatography in the re-investigation of questioned batches of insulin solutions to avoid costly bioassays, assessment of weight variation of tablets by sequential analysis, instrumental methods using quantitative infra-red measurements, gas-liquid chromatography and emission spectrography; many other examples can be quoted.

Duplication of Analyses

We have, no doubt, realised from time to time that a considerable amount of work is done by laboratories, confirming the quality of supplies received by their firm, which is repetition of work already done by the

supplier before dispatch. This practice is usual and it follows that, during the course of buying and selling, it is possible for several analyses to be done on each batch of a product.

Recent examination of the analytical records of my department for several thousands of batches of materials bought to the B.P., B.P.C., or B.Vet.C. specifications during the previous three years showed that only a few batches had been rejected. Moreover all rejections had been because of non-compliance with physical criteria only, e.g. colour, quality of solution, or presence of dirt, where the fault could be readily observed. The cost of analysis was about £15,000. Hence, avoidance of analysis would have resulted in a very impressive saving in analytical costs without concomitant disadvantage.

It will be accepted that any material offered by a reputable manufacturer has been adequately tested in their laboratories, hence it is reasonable that reputable companies should accept each other's analyses. If two firms involved in a commercial transaction were members of a group which had agreed to do this, the analytical figures relevant to the material bought could be supplied to the purchaser whose analyst need only carry out a check of a superficial kind, such as inspection, identity and simple solution to guard against dirt and adventitious contamination during packing; if these were satisfactory the consignment could be accepted on the vendor's certificate of analysis.

At an informal meeting between the chief analysts of fourteen leading pharmaceutical manufacturers, the principle of the reciprocal acceptance of analytical certificates for official products was unanimously approved, the working of the scheme to be negotiated between individual firms and to be considered as an agreement or understanding between the analysts concerned. A manufacturer would sell material with a certificate giving the batch analysis. The receiving analyst would use his discretion whether he would want to confirm any of the figures for his particular requirements. In order to provide the analyst of the purchasing firm with the means whereby the batch can be assessed for any special requirements it is desirable that the actual analytical figures should be given where such are normally determined (e.g. loss on drying, melting point, assay, etc.); the results of limit tests would not be quantitatively stated except where an actual figure is normally recorded. In all cases it is the responsibility of the receiving analyst to make what checks he thinks necessary, bearing in mind such things as the question of re-sale. Otherwise he accepts the goods on the analyses given and thus saves considerable analytical time.

Provided the scheme is operated between analysts each with respect for the other's professional integrity it is, in effect, little different from the receiving of analytical information from the analyst's own staff, which information he assesses and either accepts or requires to be supplemented with further work according to the figures presented.

Agreements of this type are now successfully operating between several of the previously mentioned firms and in all cases have been extended to cover other than official materials. It has been found that since certificates of analysis are commonly provided for overseas buyers and other customers,

the adoption of such a scheme need not impose much extra burden on clerical staff if a sensible attitude towards documentation is adopted. Of course, some firms have to provide more certificates of analysis than they receive and to meet this an added charge to recover overheads involved in clerical work might be appropriate, but this would be considerably less than a comparable analytical cost. Further problems, such as those arising from flow-line production or blended batches, have not presented particular difficulties and the scheme is well worthy of expansion.

Possible Framework for Future Legislative Control

With the contemplated revision of legislation concerning medicines, a golden opportunity is presented for re-organisation of the overall control of drugs. Concisely, the provision of standards for all drugs should be dealt with by one organisation and the control of drugs to those standards should be the responsibility of another. Drug control should follow a logical course (*a*) notification (showing therapeutic indications, toxicity, clinical trials and methods of test), (*b*) fixing of standards, (*c*) control to those standards; (*a*) and (*b*) should be regulated by the General Medical Council or a similar body and (*c*) by the Ministry of Health.

The Medical Act (1956) requires the General Medical Council to publish from time to time new editions of the B.P. which should contain "descriptions of and standards for, and notes and other matter relating to medicines, preparations, materials, and other articles used in the practice of medicine, surgery or midwifery". Although it may not have been intended as such, the present B.P. is virtually a book of standards and includes those drugs considered either most effective or of widest clinical use. With the rapid increase in chemotherapy, selection is squeezing out the drugs of natural origin, and their galenicals, and the book has become mainly a collection of specifications (i.e. a list of requirements with which the material must comply) for pure synthetic chemicals and their simple formulations such as tablets and injections, with appropriate methods of analysis. The B.P.C. includes many more drugs in common use and it controls by formulation the manufacture of a large number of more complex preparations. The Codex, also, has to be selective and a glance at the deletions with successive editions gives a clear insight into the trend in pharmaceutical usage.

Standards for many drugs are therefore already available in these publications and some control is possible by insistence on these standards, although legally they are only presumptive. Because of the time involved in compiling these books, however, new drugs intended for official recognition must wait perhaps some three years for their standardisation.

As well as the officially recognised drugs, there are other drugs on the market, too recent, too ephemeral, or of too doubtful efficacy to be included, which should be controlled for purity. This may be of particular importance for some biological materials which, when first available, are relatively impure. Such drugs are not amenable to chemical or physical control methods; a few selected drugs requiring biological

methods of test are already controlled legally by Regulations under the Therapeutic Substances Act. Hence some form of procedure should be devised to enable standards to be provided speedily for all drugs.

The obvious and ideal way would be to fix standards before a drug is placed on the market. The General Medical Council have already indicated, in their President's disclosure of the substance of evidence submitted to the Interdepartmental Working Party on Legislation Concerning Medicines, that the Pharmacopoeia Commission had made a number of recommendations which would enable the Council to publish, as approved specifications, standards for all newly introduced drugs as soon as, or soon after, they were placed on sale in the United Kingdom. No information, however, is available on how the General Medical Council mean to implement their intentions. This should not prove difficult. The standards would be available since when a new drug is marketed its analytical specification would certainly have been already worked out by the manufacturer; even now the information required to compile official monographs must rely on data provided by manufacturers or some firm using the substance. The scheme could be implemented by some central authority in a similar manner to the method already used by the Ministry of Agriculture, Fisheries, and Food in their Agricultural Chemicals notification scheme which, although primarily intended to control pesticides on their toxicity, does require methods of analysis to be provided.

In the Notification of Pesticides Scheme, firms voluntarily notify the Ministry of Agriculture, Fisheries, and Food of new toxic chemicals and formulations which they propose to introduce into agricultural practice. The procedure is intended to provide only for the safe use of chemicals and is not concerned with the approval of claims for biological uses. Information requested on notification includes the active ingredient and its proposed concentration, type of formulation, methods of analysis, proposed uses, and adequate toxicological information. The Scheme covers any product utilising a completely new active principle, i.e. a chemical not previously commercially available in the United Kingdom, and any product containing an active principle that is not new but is in a new formulation which could produce a new or increased risk. Notification is not expected while the product or proposed new use is at the stage of laboratory or small-scale trials carried out under the direction and control of the notifying firm. Care is taken to avoid the procedure being slowed down. Should consultation outside Government Service be needed no information provided by the notifying firms is disclosed to any person having a connection with any commercial interest that might make it undesirable for him to be consulted; the notifying firm is consulted before any confidential information is disclosed. Likewise reputable pharmaceutical firms would certainly be prepared to supply methods of analysis for any of their products to the Ministry of Health.

Although I am not intending here to consider responsibilities on the therapeutic value of new drugs, such a scheme could also embody this aspect of drug control in a manner analogous to the Agricultural Chemicals Approval Scheme. This scheme is intended to provide only for the

approval of claims for biological uses and products cannot be approved unless they have been through the Notification Scheme. This procedure is voluntary and a scheme for drugs could also begin on a voluntary basis; if necessary it could be made obligatory later.

Notification of new drugs should also include those imported from foreign countries. This presents no problem provided the central authority has been set up. The converse is well known, with the extensive registration requirements already in existence for our drugs overseas, where description of the drug, dosage forms, pack, therapeutic indications, and methods of analysis all have to be provided *in extenso*.

The large increase in the number of drugs used and the developments in chemical, physical, and biological methods for their examination has placed those whose duties include the control of drugs on behalf of the consumer in a difficult position. The inclusion of drugs in the Food and Drugs Act appears to have been quite fortuitous and could be ascribed to the chance that Hassall in the middle of the last century based much of his disclosure of adulteration at that time on microscopical examination, for which crude drugs and spices was an excellent field. Samples of drugs taken under the Food and Drugs Act have been confined almost entirely to a selection of popular galenicals and comparison of many recent reports on this subject with those of forty years ago shows little change. This is no criticism of the body of public analysts who do an admirable job within their sphere of activity; it is simply that, as has already been implied, pharmaceutical analysis has outgrown the general field covered by the training and practice of most of these officials. The preparations selected for testing under the National Health Service scheme have generally been limited to the more commonly prescribed standard drugs and prescriptions. But the vogue in prescribing has altered considerably in the last few years, particularly in the proportion of proprietary preparations—few of which are tested. Thus we obviously do not have full control of drugs and a change of legislation or procedure is desirable if protection of the Ministry and the public is to be obtained, both in the dispensing of prescriptions and in open sale of medicines (and these should include any cosmetic where therapeutic value is claimed).

Hence I suggest that the control of drugs should be taken out of the Food and Drugs Act and incorporated in a new Medical Substances and Preparations Act built round Sections 11, 12, and 13 of the Pharmacy and Medicines Act. For effective enforcement, legislation should provide legal status for the standards of the B.P., B.P.C., and B.Vet.C. and also for standards for any other drugs officially recognised in the procedure for provision of standards I have just outlined. It may be desirable to incorporate authority for provision of standards with that for legal enforcement of those standards in one Act. An advisory body or bodies will be required to make recommendations under the new Act.

The Pharmaceutical Society, in evidence submitted to the Interdepartmental Working Party on Legislation Concerning Medicines, has likewise recommended that the control of medicines be separated from that of

other substances and vested in the Ministry of Health. It is undoubtedly the concern of the Ministry as the chief buyer of drugs.

If the sale of drugs both for human and veterinary use is to be controlled adequately and fully, such testing must be in the hands of experts in this field. This of necessity leads to the setting up of specialist laboratories and it follows that they must be of a regional type since local authorities could not maintain them. The regional laboratories should be directly responsible to the Ministry, who through them could also maintain the National Health Service testing scheme. Even these laboratories could not adequately cope with the complete control of drugs but the load of testing could be lightened, for instance, by the method used by the Ministry of Supply where, for approved firms, the contractor's own inspection organisation is given the initial responsibility, under the Inspection Directorate, for compliance of goods with specification. The Chief Inspector so approved under the Directorate of Chemical Inspection is generally the Chief Analyst of the company and certain conditions are laid down concerning his appointment and duties.

Control should also provide against the importation of inferior drugs to wholesale users in this country for use in formulations where tests would not disclose the quality of drug used. To guard against such a contingency it should be laid down that an imported consignment of a drug must not be sold or used unless it carries a certificate of purity from a recognised laboratory in this country. The Directory of Independent Consultants issued by the Royal Institute of Chemistry could be used as a basis for recognition or, better—by parallel with the present statutory requirements for competence as a public analyst under the Food and Drugs Act—a special diploma in drug analysis from the Pharmaceutical Society or the Royal Institute of Chemistry could be demanded. This could be incorporated as a requirement of competence in the proposed Medical Substances and Preparations Act. Such a requirement would be in keeping with the suggestion already made of the need for courses in pharmaceutical analysis.

In the time at my disposal I have endeavoured to show that with the exceptionally rapid increase in the need for new analytical techniques, due to more stringent control, the added cost to the production of drugs must be considered. Other forms of expenditure in an analytical laboratory that might be reduced by investigation have been mentioned.

In my opinion, because of this rapid increase, the pharmaceutical analyst has been given insufficient time to adjust his research to the needs and if the stringency of control described is necessary a closer assessment of his precision must be made. The use of reference standards for differential analysis is almost essential if consistent results are to be obtained and such a collection is an imperative need.

Full notification of all drugs and their control is a necessity and should be incorporated in a new drug Act if the service by Pharmacy to the public is to be maintained at its present high level.

SYMPOSIUM

TOPICAL MEDICATION IN RELATION TO SKIN PHYSIOLOGY

By F. J. Ebling, M.Sc., Ph.D.

Department of Zoology, Sheffield University

The human skin has withstood topical medication from unknown antiquity to known adrenocorticosteroids. In the face of such a tradition it is no easy thing to make a critical assessment of the current situation, still less to look into the future.

The rationality of topical therapy requires not only that the medicament should be effective, but that local application of it should be better than systemic administration. For example, the local elimination of animal ectoparasites, by the use of benzyl benzoate or tetraethylthiuram monosulphide for scabies or DDT for head lice, is unquestionably effective. On the other hand, topical therapy of some fungal diseases of the skin is becoming superseded by griseofulvin, a systemic fungicide which may be taken orally (see, for example, Martin, 1959; Blank and Roth, 1959; Reiss, Kornblee and Gordon, 1960; Blank, 1960). The best mode of attack on bacterial infections of the skin is more open to debate. Pillsbury, Shelley and Kligman (1956) believe that topical therapy is preferable to systemic only in very superficial infections. Several antibiotics, such as neomycin and bacitracin, are effective, but they council strongly against all topical use of sulphonamides, penicillin, streptomycin, organic mercurial compounds and time-honoured tincture of iodine; this is, however, more because of the likelihood of induced hypersensitivity than for lack of effectiveness.

Though the variety of other traditional topical medicaments is large, I think we must admit that their efficacy is usually questionable and their mode of action, if any, unknown. Indeed, many preparations would probably be more beneficial as bland dressings with their supposed "active" ingredients left out. The necessity of water, though not too much of it, to the cornified epithelium is undoubted (Blank, 1952), particularly if the water absorbing properties are reduced in skin disorders (Flesch and Jackson Esoda, 1957).

Adrenocorticoids have within the last 10 years proved to be the most effective group of substances yet discovered for the treatment of skin disorders. Stoughton (1959) lists more than seventeen skin diseases in which oral or intracutaneous administration of such compounds was effective. In only three, namely, atopic dermatitis, nummular eczema and anogenital pruritis was topical application equally good, though there were also variable responses in other "eczematous" disorders. Nevertheless, such conditions account for about half of all cases of skin disease. Though cortisone is without significant action (Goldman, Thomson and Trice, 1952), hydrocortisone is effective as a topical agent (Sulzberger, Witten and Smith, 1953; Malkinson and Wells, 1954; Witten, 1955). Fluorohydrocortisone, in one-tenth of the concentration,

is as effective as hydrocortisone (Sulzberger, 1955). More recently other synthetic corticoids, of which triamcinolone (Rein, Fleischmajer and Rosenthal, 1957) appears to be the most potent, have been introduced for topical therapy.

Since steroids, including cortisone and hydrocortisone, are readily absorbed by intact skin (Malkinson and Ferguson, 1955; Malkinson, Ferguson and Wang, 1957; Goldzieher and Baker, 1960), the difference between the effects of cortisone and hydrocortisone is surprising. One possible explanation is suggested by the finding of Malkinson (1958) that, at skin sites from which the barrier is removed, the absorption of hydrocortisone free alcohol but not of cortisone is greatly increased. Hence hydrocortisone may more readily pass into skin in which the barrier has been damaged by inflammation. Other possibilities are that cortisone is metabolically inactivated more quickly than hydrocortisone, or that the action of the hormone may depend upon intermediate metabolic products which are readily formed in the skin from hydrocortisone but not from cortisone (Malkinson, Lee and Cutukovic, 1959; Malkinson, 1960).

How do these substances work? On a biochemical basis we do not know. But there are many experimental results indicating how various substances including steroids, may affect skin and I am going to try and review this field. I shall do so not in relation to particular chemical compounds, about which as a zoologist I know little, nor specifically in relation to skin diseases, about which I know less, but in relation to the structure and function of some of the components of the skin. I omit the apocrine glands and the condition hidradenitis, which may in part have an endocrine cause (Brunsting, 1952) and which has been systemically treated with both testosterone (Cornbleet, 1952) and hydrocortisone (Danto, 1958), and also the eccrine sweat glands. The condition miliaria or "prickly heat", according to Pillsbury and others (1956) has borne the brunt of a full pharmacopeia and the only specific treatment is to eliminate sweating. Neither shall I deal with the melanocyte nor the hair follicle, a structure of great fascination for study, but from the viewpoint of topical application unyielding, though not perhaps unrewarding.

The Epidermis

Cells are formed in the basal layers of the *stratum Malpighi* and move outwards. In the region known as the *stratum granulosum* keratin begins to be synthesised within them; the cells eventually lose their nuclei as they pass to form the *stratum corneum.*

There seems little reason to doubt that the intermediate metabolism of carbohydrates by the epidermis involves the Krebs cycle (Griesemer and Gould, 1954, 1955; Cruickshank, Hershey and Lewis, 1958), and the distribution of relevant enzymes has been investigated by, for example, Ellis and Montagna (1958) and Goltz, Fusaro, Blazejovsky and Jarvis (1959).

The metabolic processes uniquely associated with epidermis, such as keratinisation, have been reviewed by Lorincz and Stoughton (1958).

Keratins are composed of long polypeptide chains held together by cross linkages. The most important of these is the disulphide bond, formed by the oxidation of two sulphydryl groups belonging to two cysteine residues in adjacent polypeptide chains. The result is a molecule of cystine to which both polypeptide chains contribute; this reaction does not require energy but releases it. Nevertheless, according to Jarrett, Spearman and Hardy (1959), the *stratum granulosum* contains a high energy system on the evidence supplied by the distribution of enzymes. They suggest that in this region there is an active breakdown and re-synthesis of polypeptide chains, before keratinisation by cross-bonding takes place.

In the skin condition *psoriasis* there is no granular layer and the cells of the *stratum corneum* retain nuclei. Keratinisation is abnormal; there is an unusually high concentration of sulphydryl groups, suggesting that the breakdown and resynthesis of unfolded polypeptide chains has been incomplete, and the high amount of phospholipid probably is evidence of incomplete utilisation of the high energy system. The most obvious explanation of parakeratosis would appear to be a specific enzyme defect. Roe (1959) concluded that an abnormal glycoprotein accumulates in psoriatic epidermis. Because of a systemic error in sulphur metabolism this incorporates sulphur which thus becomes unavailable for keratin synthesis. Flesch and Jackson Esoda (1961) believe that a mucopolysaccharide builds up in the pathologic horny layer instead of becoming decomposed as in normal keratinisation. A similar view is held by Tickner (1961), who suggests that the psoriatic lesion arises from a failure of the union of tonofibrin fibrils due to the presence, in abnormally large quantity, of a substance produced by some metabolic block. Jarrett and others (1959) and Van Scott and Reinertson (1959), on the other hand, incline to the view that the lesion involves excessive cellular proliferation, with the result that there is insufficient time for breakdown and re-synthesis of the polypeptides, tonofibril formation is not completed, formation of the stratum granulosum does not occur, and normal keratinisation is not achieved. The question of what factors control epidermal mitosis and what substances influence it is therefore of importance.

Bullough (1946, 1950a, b) and Allen (1956) have presented evidence that oestrogens stimulate mitosis in the epidermis of the mouse. Bullough (1953) put forward the view that cell division requires energy derived from carbohydrate metabolism and that oestrogens facilitate a stage, possibly the hexokinase reaction, which is normally rate-limited. Gelfant (1959a, b, 1960a, b, c) has challenged these conclusions, maintaining that there is no concrete evidence that glucose is actually used as a source of energy for mitosis and that oestrogens have no effect. Oestrogens do not seem to affect epidermal mitosis in the rat (Carter, 1953; Ebling, 1954, 1955); it is interesting, however, that they seem to reduce epidermal thickness, suggesting that cell life is diminished and the rate of cell loss is increased. Hypophysectomy results in significant thickening of the epidermis, producing a well marked granular layer, in both female and male rats (Ebling,

1955, 1957a); this thickened epidermis can be reduced by oestrogens. Androgens, also, have been shown to stimulate epidermal mitosis in the mouse and rat (Bullough and Van Oordt, 1950; Ebling, 1957a, b).

Vitamin A causes hypertrophy of the epidermis in rats (Studer and Frey, 1952; Bern, Elias, Pickett, Powers and Harkness, 1955; Sobel, Parnell, Sherman and Bradley, 1958) and in guinea-pigs (Montagna, 1954), though Fisher and Herrmann (1957) could find no such effect after topical application to human skin. Lawrence and Bern (1958), in a very careful study of the effects of topically applied vitamin A on mouse skin, showed that the epidermal thickness was linearly related to the log of the dose, and that the increased thickness was the result of rapid epidermal proliferation.

Many agents inhibit epidermal cell division; prominent are adrenaline (Bullough, 1955; Ghadially and Green, 1957; Gelfant, 1960) and adrenal steroids such as cortisone (Ghadially and Green, 1957) and desoxycorticosterone acetate (Gelfant, 1960).

Attempts to treat psoriasis by systemic or topical application of adrenocortical steroids were not initially crowned with unqualified success. Fergusson and Dewar (1957), for example, reported inconsistent results with ACTH or prednisolone. The synthetic corticoid triamcinolone seems to show more promise. Shelley, Harun and Pillsbury (1958) reported that 36 out of 60 patients given oral triamcinolone showed an unquestionable response within a week. Symptoms were erased by 2 to 4 weeks, but reappeared when treatment was stopped. Jarrett and Witham (1961) have reported that such treatment causes reappearance of a granular layer in the psoriatic areas. Cohen and Baer (1960) found that oral triamcinolone was more effective than methyl prednisolone or prednisolone; others, while agreeing that triamcinolone is a potent drug for systemic treatment of psoriasis, point out that undesirable side effects are common and it is unsuitable for general use (Greenlee and Epstein, 1959). However, effective results from topical medication have been achieved with triamcinolone acetonide in lotion (Crowe, Fitzpatrick, Walker and Olson, 1958) or given into the lesions (Cohen and Baer, 1960; James, 1960; Readett, 1961) and by subdermal infiltration with triamcinolone diacetate (Gerard, 1960).

Jarrett and Spearman (1959) confirmed the observation of Lawrence and Bern (1958) that topical application of vitamin A to mouse tail epidermis causes formation of a granular layer and conversion of "parakeratotic" scales to flexible keratin. They concluded that it would be worthwhile treating psoriasis with a combination of local vitamin A, to promote formation of a granular layer, and systemic or topical triamcinolone to reduce epidermal mitosis. Such treatment was successful, though a full report is awaited.

Corticoid-like steroids, such as triamcinolone, clearly show promise for the treatment of psoriasis, especially if undesirable side effects can be avoided by topical application. Though histological evidence supports its rationality, it may still be premature to regard the treatment as specific. A whole range of unrelated compounds may act in a similar way. Burks and Montgomery (1943), for example, achieved formation of a stratum

granulosum and diminished parakeratosis in psoriatic lesions by the use of old-fashioned tar and ultra-violet light. It is interesting that Läuppi and Studer (1959) have been able, by means of a phenanthrene derivative, to inhibit the proliferation of the epidermis of rats which is induced by testosterone. Van Scott and Reinertson (1959) reported clearing of psoriatic lesions, with a thickened granular layer and inhibited epidermal hyperplasia, after topical application of mitotic inhibitors such as podophyllin and colchicine, mercury (20 per cent in "aquaphor"), nitrogen mustard, and liquor carbonis detergens. Methotrexate (a folic acid antagonist), 5-fluorouracil and actinomycin D were effective when given intravenously but not topically; this suggested that they may have an indirect action.

Sebaceous Glands

The sebaceous glands are truly holocrine; the cells are replaced around the periphery and the sebum is formed by the breakdown of the whole cell as it moves towards the duct. Many factors have been alleged to affect sebaceous activity, but the overriding importance of steroid hormones has been shown both in animal experiments and in human trials.

Androgens, systemically administered or topically applied, cause enlargement of the sebaceous glands of animals (de Graaf, 1943; Ebling, 1948; Montagna and Kenyon, 1949; Hamilton and Montagna, 1950; Haskin, Lasher and Rothman, 1953; Lapière, 1953). The effect seems to involve an increase in cell size as well as in cell division (Ebling, 1957). Though Shelley and Hurley (1957) failed to observe any enlargement of the sebaceous glands after implantation of testosterone into the human axilla, Jarrett (1959) showed clearly that intramuscular injection of 25 mg./day of testosterone caused a marked increase in the surface sebum in adolescent boys. Enlargement of the sebaceous glands by testosterone requires the presence of the pituitary (Lasher, Lorincz and Rothman, 1955; Ebling, 1957). It has been proposed by Lorincz and Lancaster (1957) that the pituitary contains a "sebotropic" factor.

The possible effects of progesterone are subject to some dispute. Haskin, Lasher and Rothman (1953) and Lasher, Lorincz and Rothman (1954) found that doses of 1–10 mg. daily stimulated the sebaceous glands in spayed adult rats, and stated that this effect was comparable with that of testosterone. Hodgson-Jones, MacKenna and Wheatley (1952) have shown that in man the sebum level fluctuates during the menstrual cycle, being highest in the luteal phase, and rises during pregnancy. Zeligman and Hubener (1957) have claimed that progesterone produces mild to moderate acne in women, involving slight but not statistically significant enlargement of the sebaceous glands, and Smith (1959) showed that progesterone increased the surface sebum in senile women. On the other hand, Ebling (1961), using doses of 0·1–0·2 mg. of progesterone per day for 3 weeks could find no effect in either intact or spayed, immature or adult, female rats, and Jarrett (1959) found that the amount of surface sebum was unaltered by treating adolescent men and women with progesterone.

Adrenocorticoids have been reported by Castor and Baker (1950) to reduce the size of sebaceous glands when applied locally, though Haskin and others (1953) achieved only a slight effect by the injection of cortisone. Systemic administration of ACTH, similarly suppresses the glands, according to Baker, Ingle, Li and Evans (1948), but Haskin and others (1953) found that this treatment caused glandular enlargement. In man, Strauss and Kligman (1959) found that enlargement of sebaceous glands was induced by ACTH in a proportion of males and females, though only 2 out of 6 treated with hydrocortisone showed such a response. It is difficult to reconcile all these results, though the suggestion of Haskin and others (1953) that ACTH causes production of adrenal androgens could explain a difference between its effect and that of corticoids.

Oestrogens, by general agreement, cause a reduction in the size of sebaceous glands (Hooker and Pfeiffer, 1943; Ebling, 1948, 1951). In rats, the effect of oestradiol-17β appears to be brought about by a more rapid disintegration of the sebaceous cells, and a reduced cell production is not necessarily involved (Ebling, 1954, 1955). Indeed, by combining oestradiol and testosterone, the incidence of mitoses can be raised without increasing gland size (Ebling, 1957). The overall effect of oestrogens is one of reduced sebum production, as demonstrated clearly by Jarrett (1955) in human patients given stilboestrol. It seems likely that the sebaceous cells have no time to differentiate fully before they are shed.

The effect of oestrogens is independent of the presence of the pituitary (Ebling, 1955), and it can be produced locally (Lapière, 1953). Natural oestrogens such as oestrone and oestriol are also effective, as are a number of synthetic steroids of low oestrogenic potency such as 16-epi-oestriol and oestradiol-17α (Bullough and Laurence, 1960).

These facts might suggest a clear endocrine etiology for skin disorders such as acne vulgaris and seborrhoea, which involve enlargement of the sebaceous glands, as well as a rational approach to systemic or topical therapy. Lipman Cohen (1941) drew attention to the probable endocrine background of acne, and Hamilton (1941) stated clearly that male hormone was a prime factor, a view endorsed by Rony and Zakon (1943). But the idea that acne results solely from a high production of androgens may be too simple. Some authors, for example Aron-Brunetière (1953), have proposed that the essential cause is an increased androgen : oestrogen ratio. Moreover, the demonstration that androgens are without effect in the absence of the pituitary suggests that a hypophyseal hormone may be involved in the etiology of acne. The condition may perhaps occur in the male because a rising production of androgens during adolescence overlaps some hypophyseal activity which later abates. Lorincz and Lancaster (1957) believe that the hypophyseal factor has a separate identity from known pituitary hormones.

Haskin and others (1953) have suggested that adolescent seborrhea and acne in the female is the result of luteal progesterone and not of adrenal androgens. Since in experimental animals progesterone has been shown to enlarge the sebaceous glands only in relatively large doses, we cannot be sure that its action is not dependent on prior conversion to androgens.

Such a process might also explain its effect in senile women reported by Smith (1959). If this were so, a direct role of progesterone in the etiology of female acne would be precluded.

Amelioration of acne after treatment with oestrogens has been reported by a number of authors. Lawrence and Werthessen (1942) successfully used orally administered oestrogens, and Jarrett (1955) found that 3–5 mg. of oral stilboestrol per day had a beneficial but temporary effect. The question arises of how far topical application is of equal value. Whitelaw (1951) applied 1·25 mg. of sodium oestrone sulphate daily in an ointment to adolescent males and females and noticed great improvement in more than half of them within 6 months, without any side effects in the males. More recently, Peterkin (1959) has reported that application of a lotion base containing 1 mg./ml. of epioestriol improved 73 per cent of acne patients within 2 months, compared with only 45 per cent when the base alone was used.

The Dermis

The connective tissue of the dermis consists mainly of a complex association of a metabolically inert protein, called collagen, and mucopolysaccharide. The collagen is traditionally observed as bundles of fibres and the mucopolysaccharide as the semifluid amorphous ground substance, though Jarrett (1958) suggests that the histological appearance of fibres and spaces may be an artefact of fixation. In addition the dermis contains elastic fibres, vascular beds at various levels, fibroblasts which secrete the unpolymerised tropocollagen from which the collagen is formed, mast cells, melanocytes, macrophages, lymphocytes and other leucocytes (Montagna, 1956).

In many skin diseases inflammation occurs in the dermis, and similar changes can be brought about by such diverse stimuli as bacterial invasion, thermal injury and frostbite. "Inflammation" is an overworked word and an undefined process though two features seem to be of special importance in its pathology, namely increased capillary permeability to protein and the emigration of leucocytes. A number of endogenous substances will increase capillary permeability. Histamine is produced by the mast cells in man and most other mammals; 5-hydroxytryptamine (serotonin) appears to be produced by mast cells in rodents, but by blood platelets in man (Spector, 1958; Schachter, 1960). Bradykinin and kallidin are polypeptides which are released from a plasma globulin by an enzyme kallikrein (Cormia and Dougherty, 1960), and there are other similar substances, for example, "leukotaxine" prepared by Menkin (1951a).

The release of histamine, which can be induced in man by intradermal injection of various specific histamine liberators, produces "flare" and "wheal"; subcutaneous injection of such substances produces erythema, pruritis and oedema. But how histamine is held in the cell or released cannot be satisfactorily explained; antihistamine drugs are mostly synthetic analogues of histamine which do not affect histamine release but act by competitive inhibition. Histamine release after injury of skin is

almost immediate and of brief duration, and there is evidence that inflammation is maintained and sustained by a mechanism insensitive to antihistamine measures (Spector, 1960). Nevertheless, abnormally large amounts of excreted histamine have been shown in some diseases, for example, urticaria pigmentosa (Demis, Walton and Higdon, 1961). In atopic dermatitis, also, skin histamine levels have been shown to be above normal, though Johnson De Oreo, Lascheid and Mitchell (1960) concluded that this did not justify the conclusion that histamine was responsible for or of major significance in the cutaneous alterations.

Though steroids have been reported to have many different dermal effects, there is little precise information on their mode of action. In a careful paper on "Histological effects of hydrocortisone in the skin of man", Goldman (1955) had to conclude that these studies "do not suggest any mechanism for the local suppression of inflammation. Our techniques are at present too crude. . . ." It may be of value, nevertheless, to try and summarise the existing clues.

Steroids may act on the collagen-mucopolysaccharide complex. Oestrogens have been shown to increase the amount of intracellular water in the skin of mice, probably by increasing the amount of ground substance (Cooper and Schmidt, 1957a, b). Corticoids appear to have an opposite effect. Hydrocortisone ointment applied to man (1 per cent twice daily for 1–8 months) or oral cortisone or prednisone, or ACTH, caused progressive atrophy of collagen fibres as well as disappearance of interfibrillar mucopolysaccharides, dissociation of elastic fibres and atrophy of fibroblasts (Mancini, Stringa and Canepa, 1960). A decrease in the ratio of hexosamine (derived from mucopolysaccharide) to collagen in biopsies from the buttocks after 2 weeks systemic medication with prednisone, methylprednisone or triamcinolone has been reported by Wright, Sobel and Nelson (1960). In the rat, the uptake of ^{14}C and ^{35}S by mucopolysaccharide constituents is inhibited by cortisone or hydrocortisone (Schiller and Dorfman, 1957).

The "anti-inflammatory" action of adrenocortical steroids is undisputed, but the mechanism is debatable. There is evidence that the mast cells themselves are inhibited; according to Asboe-Hansen (1957, 1958) adrenocortical compounds cause clumping of the granules, a slower uptake of ^{35}S and reduced histamine secretion. Vacuolation and disruption of mast cells with release of granules, as well as an increase in the number of binucleate cells, is induced in human tissue by cortisone (Bloom, 1958). ACTH reduces the number of circulating blood mast cells in the rabbit (Boseila, 1958).

On the other hand, Menkin (1951a, b) states that cortisone suppresses the increase of permeability due to the liberation of polypeptides ("leukotaxine"). Dougherty and Schneebeli (1955) and Scott and Kalz (1956) consider that adrenocorticoids act, not by inhibiting the production of the inflammatory stimulus, but by interfering with its action. Dougherty and Schneebeli (1955) produced inflammation in the skin of adrenalectomised mice by a variety of methods, and showed that antiphlogistic steroids prevent destruction of the fibroblasts and reduce the numbers of

invading leucocytes and macrophages, as seen in loose connective tissue spreads. By the use of ^{14}C-labelled hydrocortisone and autoradiography they showed that the steroid actually accumulates within the fibroblasts; such cells resist destruction.

Spector (1958) has pointed out that there are two hypotheses about the mode of action of cortisone and hydrocortisone on capillary permeability. Either they prevent the antigen-antibody combination from exerting its effect on the capillary wall, or they cause a general depression of the reactivity of the capillary wall to stimuli which increase permeability. The evidence suggests that there is some truth in both hypotheses. Marks, Smith and Cunliffe (1961) have suggested that salicylates, also, act by preventing antigen-antibody combinations from exerting their effects on the capillary wall.

An effect on the blood supply in experimental chambers made in rabbit ears has been observed by Ebert and Barclay (1952). Cortisone brought about increased vascular tone in the arterioles and reduced sticking of leucocytes to the arteriolar endothelium.

The anti-inflammatory action of cortisone is inhibited by desoxycorticosterone (Dougherty, 1954). It is interesting to note that, in tissues which are specially sensitive to them, such as the uterus and vagina, oestrogens produce all the features of inflammation, including leucocyte emigration.

Corticoids are usually regarded as effective antipruritic agents, though experimental evidence to support such an action is sometimes conflicting. Cormia and Kuykendall (1953) found that, though antihistamines, analgesics such as aspirin and codeine, and sedatives all raised the threshold concentration of histamine needed to produce a recognisable pruritis, intramuscular cortisone had only a very little effect. Frank (1958) found that neither hydrocortisone free alcohol nor hydrocortisone diethylaminoacetate had any effect when applied after histamine, but both steroids shortened the duration of pruritis when applied 2 hours previously. They also had a significant antipruritic effect, as compared with a blank vehicle, in patients, though less than half benefited even with the highest concentration (0·5 per cent) used. Macris, Blank and Beecher (1959) trained investigators to record the duration of experimental pruritis after the application of cowhage (pods of *Macuna pruriens*). They reported that calamine lotion, ointments of menthol, xylocaine, nupercaine or hydrocortisone, together with various vehicles and placebos had no effect on the duration of pruritis.

Conclusions

Some traditional topical medicaments are becoming discarded as valueless or are being superseded by more effective systemic remedies. In addition, the use of steroids, especially those of the adrenal cortex or their synthetic analogues, is opening up new possibilities of local therapy. In spite of the development of some preparations that are "anti-inflammatory and antipruritic" we do not know exactly how such substances work, any more than we understand the pathogenesis of most

skin diseases. Steroids may affect the epidermis and its appendages as well as the dermis.

A decade or so ago the skin could reasonably have been regarded as a neglected organ. This is no longer true; skin physiology and experimental dermatology, as well as steroid chemistry are developing rapidly and we may expect further improvements in skin therapy.

In conclusion I turn again to antiquity. Moses (1451 B.C.) reported that, for disobedience of the commandments, "The Lord will smite thee with the botch of Egypt, and with the emerods, and with the scab, and with the itch, whereof thou canst not be healed". Later we learn the reason for the lack of therapeutic measures: "thou shalt not anoint thyself with oil; for the olive shall cast his fruit".

Without wishing to imply that I accept the given reasons, I observe that the scab, itch and botch are still with us. But the olive does not cast his fruit; we have oil, and we have discovered even better things than oil with which to anoint ourselves. In case the opening of my paper should have appeared too sceptical, I end it by making clear that I think it proper and profitable for the search to continue.

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SKIN MEDICATION

By A. Jarrett, M.B., F.R.C.P.

Department of Dermatology, University College Hospital Medical School, London W.C.1

In the previous paper by Dr. Ebling the normal anatomy and physiology of the skin and its appendages have been described. It is the object of this paper to discuss some of the ways in which skin function can become abnormal, and to evaluate critically the therapeutic means we have at our disposal to correct these pathological changes.

Abnormalities of the Epidermis

Ebling has already described how normal keratin is formed from epidermal cells by way of a granular layer, the stratum granulosum. This normal state of affairs can be interfered with in two ways. First there may be a failure to form a granular layer, and secondly this layer may become abnormally thickened. Both result in the production of abnormal keratin.

Absence of the Granular Layer

In this disorder there is a failure to form a granular layer and this is often associated with an increase in thickness of the epidermis; this combination occurs characteristically in psoriasis. It also occurs in other conditions in which there is increased or abnormal activity of the epidermal cells; thus it is seen in most types of cancerous and precancerous conditions of the skin. As already stated the keratin formed by this disordered epidermis is itself abnormal. It has different physical characteristics in that it forms large scales that flake off. It is this scaling that the patient sees, and is the reason for his consulting his doctor.

There are also changes in the keratin that are recognisable under the microscope. Nuclear remains are present in the substance of the keratin and this is known as parakeratosis to distinguish it from normal keratin. In addition there are histochemical alterations; for example, there is an increase of phospholipids and of protein bound sulphydryl groups (Braun-Falco, 1958, 1959; Jarrett, 1959).

In our discussion on treatment we will take psoriasis as the example of this type of disordered keratinization. The malignant conditions giving rise to this type of abnormal keratinization require special treatment either by surgery or by irradiation. The latter form of treatment will be briefly evaluated later in this paper.

Increase of the Granular Layer

This is much rarer than an absence of the granular layer. Nevertheless an abnormal keratin is produced which may also appear scaly, although the scales are not so large as in psoriasis. It is seen most often in lichenification of the skin caused by chronic irritation and rubbing. It is also

present in the relatively rare disease called lichen planus. The histochemical changes in the keratin and of the epidermal cells are not so well known as in psoriasis.

Blistering Diseases of the Skin

Many skin diseases are capable of producing blisters. These are formed either as a result of damage to the epidermal cells, or by the lifting up of the whole epidermis by an accumulation of fluid at the dermo-epidermal junction. The commonest condition causing vesiculation of the skin is eczema. Here the epidermal cells are damaged, break down, and form little microscopic collections of fluid which often rupture onto the surface and produce a weeping eczema. During the healing phase the epidermis regenerates, and there is an increase in the epidermal cell activity resulting in the formation of a parakeratotic keratin. This stage is often known clinically as scaly or dry eczema. This abnormal keratin is replaced later by normal keratin after the re-establishment of a granular layer.

There are several other diseases which give rise to blistering; among these are included pemphigus, dermatitis herpetiformis, and erythema multiformae. Pemphigus exists in several forms, and in the past was a fatal disease. Erythema multiformae is often a result of sensitisation to internal drug, or food allergen.

The Value of Therapy for these Epidermal Disorders

Psoriasis

I will take this disorder as a typical example of abnormal keratinization of the parakeratotic type.

Countless remedies have been recommended for its treatment. The genuine value of most of these is open to serious doubt. Of the more effective local applications one may list the following: crude coal tar 1–10 per cent, dithranol 0·05–0·5 per cent, and local irradiation with ultraviolet light either alone or combined with crude coal tar as in the Göeckerman régime (1931).

Those of more doubtful value include salicylic acid 0·5–5 per cent either alone or combined with other medicaments, ammoniated mercury ointment with and without solution of coal tar and numerous others. I think it is fair to say that none of these substances has any known rational basis for its effectiveness, or otherwise.

Recently we have been able to demonstrate the formation of a granular layer in mouse tails where none previously existed, after the local application of vitamin A. This vitamin therefore specifically generates a granular layer. We thought that this would be of great value in the treatment of psoriasis. Vitamin A, however, has another effect in that it induces hyperplasia of the epidermis. This is obviously undesirable in psoriasis as the epidermis is already active and greatly thickened. We were able to control this increase in activity caused by vitamin A by the use of local and systemic steroids. The most useful steroid was triamcinolone, and this had already been used alone with success in the treatment

of psoriasis (Shelley, Harum and Pillsbury, 1958). However, the dosage required was often great and the complications arising from its over-enthusiastic use caused it to fall into disrepute. The combination of the vitamin with triamcinolone in an oil-in-water emulsion proved successful in the treatment of psoriasis (Jarrett and Spearman, 1959b) and further clinical trials are still in progress.

It is of interest that the vitamin must be used in a water-solubilised form; the oil soluble preparations are of little or no value. This is an intriguing observation since there is some basis for the belief that oil soluble substances are better able to penetrate the epidermis than those dissolved in water.

Thickened Granular Layer

Lichenification. This is usually due to chronic pruritus, and is the result of constant scratching. The only really effective means of control are local corticosteroids, and either Grenz or X-irradiation. Other local treatments including menthol, camphor, phenol, and anaesthetic ointments such as benzocaine or cocaine are usually either useless or frankly dangerous because they may produce a contact dermatitis.

Lichen planus. I know of no specific treatment for this condition. Those suggested in most text books are valueless and only play for time until the patient makes a spontaneous recovery. In very severe cases it is worthwhile to give a course of systemic corticosteroids or ACTH, but there may be a relapse on discontinuing therapy.

Blistering Diseases

Eczema

This is one of the commonest skin diseases, and one for which endless remedies have been suggested. It can usually be taken that the number of treatments recommended for any particular condition is in the inverse ratio to the value of any one of them: eczema has more treatments than any other skin disease.

In the early stages of weeping eczema, soaks certainly make the patient more comfortable. These may be saline soaks, lead lotion, potassium permanganate, or even calamine. Probably the water is the most useful ingredient; because of its latent heat of vapourisation it cools the skin and reduces the vascular dilatation; it also prevents the dressing adhering to the affected area. Later in the dry scaly stage, zinc paste, zinc paste and ichthammol, tar ointments as for psoriasis, and of course the local corticosteroids can be employed.

At this point it is perhaps worthwhile to mention zinc paste. It is possible that zinc can substitute for magnesium in the epidermis. It appears that magnesium is required for proper keratin formation; it acts as a catalyst in the formation of disulphide linkages from the amino-acid cysteine (Neurath and Bailey, 1954). Further experimental work is required to ascertain whether zinc can substitute for magnesium and therefore act as a catalyst for the formation of disulphide linkages. If this is so then a rational role for the use of zinc in skin diseases can be substantiated.

Pemphigus

In the past this was a fatal skin disease. If the patient survived the first attack he usually succumbed to the second or the third. The advent of steroids has greatly changed this prognosis. A patient can be controlled with steroids during the acute phase of his disease; the dosage required at this stage is often very high. The dose can then be reduced during the succeeding remission. In this manner it is often possible to keep the patient alive through several attacks and in some cases until a natural cure has been attained. Supportive measures are also required in that protein loss must be made good, and skin sepsis controlled with antibiotics (Nelson and Brodey, 1955).

Dermatitis herpetiformis. This is an example of a disease the cause of which we do not know and one which we can control with therapeutic agents whose action we do not understand. It is a wonderful example of successful empirical therapy. This relatively rare disease is controlled with small doses of sulphapyridine (0·25–1·0 g. daily) or by dapsone given in doses of 25 to 100 mg. a day. The mode of action of these two drugs in this condition is entirely unknown (Alexander, 1955; Morgan, Marsden, Coburn and Mungavin, 1955).

Infective Conditions of the Skin

Pyococcal Diseases

During the past 25 years there have been enormous strides in the control of infective conditions of the skin. With the advent of sulphonamides and antibiotics the control of these conditions has become greatly improved. We are now able to deal adequately with such conditions as impetigo, ecthyma and secondary infected eczema.

Carbundles and boils are no longer the danger they were only a few years ago. Nevertheless these conditions may be recurrent and the general health of the patient has an important part to play in their permanent cure.

Tuberculosis of the Skin

This is another success story—calciferol, isonicotinic acid and streptomycin have greatly improved the prognosis of the once dreadfully mutilating diseases lupus vulgaris and scrofuloderma.

Fungus Infections

Until very recently microsporum ringworm of the scalp in children was a real problem and necessitated X-ray epilation, which occasionally caused permanent alopecia. Local therapy was virtually useless and the loss of time from school often serious.

Modern therapy with griseofulvin has given us a relatively safe method of oral treatment and requires only the simplest of local medication. This drug has also greatly improved the treatment of favus, ringworm of the skin and of the nails. Its precise mode of action is uncertain; it is fungistatic, not fungicidal. The drug is taken into keratinized structures such as hair, nails and epidermis making them resistant to invasion by the

fungus. The infected hair or nail keratin can be mechanically removed, and the new unaffected structures allowed to grow. With epidermal infections the infected keratin is removed by the normal shedding process (Williams, Marten and Sarkany, 1958; Hargreaves, 1960).

Tropical Diseases

Diseases like cutaneous leishmaniasis, and amoebiasis of the skin are amenable to modern antiprotozoal drugs, and therefore their treatment is much more successful.

Dermal Disorders

Under this heading I am including a small group of unrelated disorders which have become amenable, at least to some extent, to modern treatment whether or not the rational basis for their success is known.

We have reason to believe that the dermis exists in the living skin as a gel and not as a network of fibres and blood vessels surrounded by fluid. The fibres appear to be a fixation artefact; they are formed by the polymerisation of the parent monomer by chemical fixatives (Jarrett, 1958).

Urticaria

This is a common skin disorder and one which can be controlled by modern therapy. Although a cause for this type of vascular reaction should always be sought, we are often forced to control the condition empirically with antihistamine drugs. These substances act as blocking agents to the histamine produced by the abnormal vascular reaction. By the use of these substances cases can often be kept symptom free until a spontaneous recovery occurs.

Lupus Erythematosus

No specific treatment for this condition is available. Nevertheless certain of the modern antimalarial drugs such as mepacrine and chloroquin are of great value although their mode of action is unknown. In the disseminated form steroids are life saving.

Scleroderma

Again there is no known specific remedy. The hormones, relaxin and oestrogens have been tried on the rational basis of altering the physical state of the collagen.

Relaxin is a hormone obtained from the ovaries of pregnant sows. In late pregnancy this hormone causes softening of the pelvic ligaments and this allows greater pelvic mobility during delivery. It is reasonable to suppose that this hormone alters the physical state of connective tissues in that it causes depolymerisation. This would theoretically improve the state of the skin in scleroderma. The results obtained with this compound have been generally rather disappointing (Evans, 1959). Steroids are of doubtful value in this condition.

Granuloma annulare. This condition is often greatly helped by vitamin E and by local injections of hydrocortisone.

Disorders of the Sebaceous Glands

Under this heading the problems of acne vulgaris and rosacea will be considered. Acne is a common disease that occurs at puberty and is associated with increased activity of the sebaceous glands.

Sebaceous glands are stimulated by androgenic hormones such as testosterone and suppressed by oestrogenic hormones. These effects have been demonstrated in the rat (Ebling, 1948, 1955, 1957, 1961) and in human patients (Jarrett, 1955, 1959a).

It is therefore reasonable that oestrogenic hormones should be used to reduce the increased sebaceous activity in cases of acne. Systemic treatment with stilboestrol has proved most useful in the control of this condition. Courses of short duration with moderately heavy dosage (3 mg. daily for 21 days) often succeed in producing remarkable improvement. Local oestrogens can be used but their effect is generally not satisfactory. The mode of action of oestrogens is uncertain. It may have two sites of action, the pituitary, and the sebaceous glands themselves. Ebling (1955) has pointed out that it is possible to demonstrate the inhibitory effect of oestrogen in hypophysectomised animals. On the other hand, a pituitary hormone, or hormones, is necessary for the stimulating effect of androgens to be manifested (Lasher, Lorinez and Rothman, 1955; Ebling, 1957). It is therefore possible that oestrogens as well as acting locally on the sebaceous glands also have an inhibitory action on the pituitary.

Acne Rosacea

This is a similar condition to acne vulgaris occurring mainly in women at the menopause. There is a marked vascular dilatation in the disorder which is not present in common acne. Oestrogens are of value in rosacea, but they are nothing like so effective as with acne vulgaris.

Physical Methods of Therapy

Under this heading can be included surgical procedures, irradiation with X-rays, Grenz rays and ultra-violet rays, diathermy, cautery, and the local application of carbon dioxide snow. All these methods have their place in dermatological treatment. With the exception of the three types of irradiation all these methods cause destruction of the tissues. Irradiation of the skin with X-rays or Grenz rays (these are X-rays produced at very low kilovoltage) are used in the treatment of several benign skin disorders such as eczema, neurodermatitis, and chronic irritating conditions. They are of great value, but their mode of action is not well understood. X-rays are of course of paramount importance in the treatment of skin malignancies. These include the malignant reticuloses as well as squamous cell and basal cell carcinomas.

The other physical methods will not be discussed here as they are of little interest to the present appraisal.

Ointment Bases

The problem of ointment bases is difficult and complex, and little is known of the relative therapeutic values of the different bases available.

The nature and penetration of therapeutic substances from their ointment bases was reviewed by Goldsmith (1954). He points out the confusion between penetration, absorption, and drug release; these terms have been used indiscriminately. He suggests that "penetration" should mean entry into the skin, and "absorption" should mean systemic distribution through the blood stream. Whereas drug release from the ointment base although essential to enable either penetration or absorption of the active agent does not imply that either have actually taken place.

Penetration of normal skin probably has no relation to the penetration of diseased skin. Moreover each type of skin disorder probably alters the penetration in a different manner either making a particular medicament from a particular vehicle more or less easily available to the epidermal cells.

It has already been mentioned that in the case of vitamin A the water soluble form appears to be more active than the oil soluble form. It may be in this case that there is better skin penetration, or it may be that the vitamin is more active in its water soluble state. Much more work is required on the penetration of the skin and to this end the use of radio active isotopes are proving of great value.

The problem of the preparation of ointments, creams and lotions, and their relative merits is discussed by Hadgraft in another paper.

Conclusion

During the past three or four decades there has been considerable progress in the treatment of skin disorders, but this has been due to a relatively small number of new drugs. These modern powerful agents exert their effects on the epidermis itself or upon the organisms attacking the skin. In the past for all, and at the present time for many skin diseases, a galaxy of therapeutic remedies are suggested. Many of these are either completely valueless or of doubtful use.

Without doubt the main advances have been due to the introduction of antibiotics and corticosteroids. This has enabled the competent dermatologist using these compounds in reasonable dosage and with skill, to control and often cure conditions that were not amenable to therapy in the past.

The problem of ointment bases and the penetration of the active substances into the skin is complex. One, however, should not use a base in which the active principle is tightly held, and therefore is not released from its vehicle.

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PHARMACEUTICAL FORMULATION IN SKIN MEDICATION

By J. W. Hadgraft, F.P.S., F.R.I.C.

*Pharmaceutical Department, Royal Free Hospital, London, W.C.*1

Numerous criteria have been listed as ideal properties of dermatological bases (Mumford, 1940, Chamings, 1943) but no one substance or preparation will meet all requirements. It is now possible to formulate a range of preparations from which the dermatologist can make a selection for the particular need he has in mind. A skin preparation should not contain ingredients likely to irritate the skin or aggravate the condition under treatment. The vehicle should not interfere with the activity of the medicament or with normal skin function unless the condition under treatment requires that there should be some modifying influence. The preparation should be pharmaceutically compatible, cosmetically acceptable, easily applied to the skin surface, stable under normal conditions of storage and should not support the growth of micro-organisms. The formulation should be kept as simple as possible since the fewer ingredients present, the less likelihood there is of introducing potentially skin sensitising substances or of interfering with the activity of the medicament.

The pharmaceutical aspects of dermatology have recently been discussed by Van Abbe (1959) who drew attention to the need for more precise knowledge of the effects of topically applied preparations and for better correlation of the physicochemical properties of the materials used in formulation and skin physiology. Many preparations commonly used are traditional and, although the broad requirements for the different types of vehicles are defined, there are no clear clinical indications for the more subtle variations which can be achieved by pharmaceutical formulation.

In the acute inflammatory stages of disease, simple aqueous lotions may be used in the form of wet dressings. They have a cooling effect on the skin, do not impede drainage and are useful in the removal of crusts and debris. Lotions containing an insoluble solid, which may be of value in the treatment of subacute conditions, combine the cooling effects of an aqueous solution with the protective effects of a powder which remains on the skin surface.

Ointments are useful for the application of insoluble and oil-soluble medicaments. They leave a greasy film on the skin, inhibiting the loss of moisture and encouraging hydration of the keratin layer. They may be of value for dry scaly lesions in which there is a need to improve the suppleness of the skin. Pastes contain a high proportion of powder dispersed in a fatty base. They combine the properties of powders, absorbing exudate, and ointments, providing a greasy protective film on the skin.

Creams combine the characteristics of the lotions and ointments. They contain water which evaporates to produce a cooling effect and the greasy film remaining on the skin surface possesses the properties of ointments.

It is the purpose of this paper to describe some of the materials used to formulate dermatological preparations and to deal with a few of the pharmaceutical problems encountered in their use.

Lotions

Lotions are usually simple aqueous solutions or suspensions which present little difficulty in formulation. Water-soluble medicaments are probably best applied to the skin in this form but it may be necessary to add other ingredients to modify the behaviour of the lotion on the skin. Methylcellulose or sodium carboxymethylcellulose together with glycerol may be added to form a water-soluble plasticised film which will help to localise the effects of the lotion and hold the active medicament in contact with the affected area.

Solubility problems may arise in the formulation of lotions. Hydrocortisone, for example, may be formulated in liquid preparations using a combination of cetomacrogol and self-emulsifying monostearin as the suspending agent. If, however, it is required in true solution this can be achieved by using a water-miscible vehicle such as liquid macrogol (Collard, 1961).

Suspending agents are used to promote the dispersion of insoluble powders in lotions. Bentonite is used for this purpose in calamine lotion but may produce thixotropic suspensions which are difficult to pour and froth excessively on shaking. Armstrong and Fenton (1954) have suggested the addition of sodium citrate to overcome these difficulties.

More investigations of suspending agents for this kind of lotion are needed. They compare badly with many cosmetic preparations of a similar character. A new concept has recently been advanced by Meyer and Cohen (1959) who have related suspending ability to the rheological characteristics of the aqueous phase. Most hydrophilic colloids (macrogols, sodium carboxymethylcellulose and methylcellulose) exhibit pseudoplastic properties and prevent the settling out of suspended particles by increasing the viscosity of the continuous phase. Substances which exhibit plasticity and show a critical yield-value when examined in a rotating cylinder viscometer may produce permanent suspensions, irrespective of their apparent viscosity. Two hydrophilic colloids have been found to have such characteristics. Tragacanth mucilage, prepared by homogenization in which it is exposed to high shear, exhibits a critical yield-value and produces permanent suspensions. A synthetic carboxyvinyl-polymer (Carbopol 934) possesses similar properties. Meyer and Cohen have suggested that materials for preparing suspensions should be selected by determining the ratio of yield-value : apparent viscosity and point out that if this value is high, permanent suspensions with maximal flow properties may be formulated.

Reviewing the factors influencing the performance of gums in cosmetic formulations, Levy (1959) also deals with the effect of homogenisation on tragacanth solutions and points out that it produces a rapid hydration of the gum. By controlled homogenisation, tragacanth solutions possessing a range of stability characteristics may be obtained.

Other ingredients in the preparation may influence the performance of suspending agents. Levy and Schwarz (1958) have found that sorbitol and glycerol lower the gel point of methylcellulose so that preparations combining these substances may become cloudy under warm conditions and change from fluid, easily-pourable liquids into opaque, semi-solid gels. Propylene glycol, on the other hand, raises the gel point of methylcellulose. Bolliger and Muenzel (1958) have also found that the addition of polyhydric alcohols to solutions of hydrophilic gums increases their viscosity and, if the concentration is sufficiently high, interferes with hydration of the gum, and an otherwise pseudoplastic system may become plastic.

Ointments

The division of ointment bases into emollient and protective types can no longer be maintained in the light of the recent findings of Blank (1952, 1955) and of Peck and Glick (1956). Oils and waxes do not soften callous tissue and the effect of ointments on the pliability of the skin depends upon their ability to form a water-insoluble film on the stratum corneum thus reducing the rate of loss of moisture from the skin. Hydration of the keratin layer by moisture from the underlying tissues is thus encouraged and it is for this reason that the skin is rendered more pliable.

Comparative studies by Powers and Fox (1957, 1959) of the effect of lotions and cosmetic creams on the water content of the skin have indicated that soft paraffin produces the greatest reduction in loss of moisture whilst anhydrous wool fat is the next most efficient. A number of surface-active agents actually increase the rate of moisture loss from the skin but this would not necessarily apply when they are combined with fatty materials in ointment bases. Glycerol, a traditional emollient, and propylene glycol also increase the loss of moisture from the skin. But the formation of an occlusive film may cause an excessive retention of water in the keratin layer and have other undesirable effects. Blockage of the hair follicles may provoke an inflammatory reaction causing folliculitis and an occlusive film may produce localised heating of the affected area.

Mineral Oils

Mineral oils such as soft paraffin and liquid paraffin are bland, inert preparations which are not absorbed by the skin (Harry, 1941). They have the advantages of chemical stability and do not react with the substances incorporated into ointments.

According to Meyer (1935), soft paraffin consists of a colloidal system containing mineral oil dispersed in a solid wax, the dispersion being stabilised by the presence of an amorphous constituent. The composition cannot be clearly defined and varies in consistency. Hard paraffin is a mixture of solid hydrocarbons with a crystalline structure which renders it difficult to incorporate with oils, unless melted and vigorously stirred during the cooling process. The effect of the variable consistency of soft paraffin may be minimised by combining it with liquid paraffin and hard paraffin as in paraffin ointment.

A range of proprietary mineral waxes are now available which have distinct advantages over hard paraffin. They have been described as "microcrystalline" or "amorphous" waxes and because of their internal structure they are superior to hard paraffin as blending agents. They produce homogeneous mixtures with mineral and vegetable oils and with synthetic esters of fatty acids (Hadgraft and Wolpert, 1960). To produce a satisfactory gel with liquid paraffin it is necessary to have a crystalline wax like hard paraffin combined with an amorphous wax, thus simulating the composition of soft paraffin. Such combinations exhibit thixotropic properties, the viscosity being reduced if the mixture is subjected to milling at high pressure (Mutimer, Riffkin, Hill and Cyr, 1956).

Polyethylene-Mineral Oil Combinations

Examination of the properties of wax-thickened mineral oils has suggested the use of hydrocarbon polymers as gelling agents for mineral oil. A proprietary base, developed on this principle, is prepared by dissolving polyethylene in mineral oil and rapidly cooling the solution by pouring on to a water-cooled metal surface. Under these conditions, supercooling occurs and the polyethylene is very rapidly precipitated in a colloidal form. The ratio of crystalline to amorphous resin produced in the final gel depends upon the molecular weight of the polymer (21,000) and the rate of cooling (about 10° per second). The ointment bases obtained are non-reactive, have reliable consistency and show little variation in viscosity over a range of temperature from 5° to 45°. Although available in America, these bases are unobtainable in this country except in proprietary ointments (U.S. Patents 2,627,938; 2,628,187; 2,628,205).

Wool Fat and Derivatives

Wool fat is more readily absorbed by the skin than are the paraffins and may be used in ointments when deeper penetration is desired. It shows freedom from rancidity and readily absorbs water to form stable water-in-oil emulsions. Its main disadvantages are its odour, stickiness on the skin and liability to show surface disolouration. Some of these disadvantages are overcome by combining wool fat with the paraffins as in simple ointment.

The use of wool-alcohols was discussed at the first Symposium Session of this Conference in 1947 (Hadgraft, 1947) and it need be mentioned only briefly here. Wool-alcohols may be combined with the paraffins to produce hydrophilic bases like ointment of wool-alcohols. But it has a number of disadvantages since it is a mixture of uncertain composition and is not readily standardised. On storage, it is liable to surface oxidation with resultant loss of its emulsifying power. The autoxidation of wool alcohols during storage at room temperature has been investigated by Clark and Kitchen (1960a) who have shown that it may be effectively inhibited by the addition of 500 p.p.m. of butylated hydroxyanisole.

In recent years, other derivatives of wool fat have become available. Hydrogenation produces a white solid wax melting at about 50° and containing hydrogenated methylsterols with dihydrocholesterol and

saturated alcohols. Hydrogenated wool fat may be combined with mineral oils and an ointment base containing 70 per cent of the wool fat preparation with 30 per cent of liquid paraffin has been suggested (Fayaud, 1953). It spreads smoothly on the skin surface, producing a less greasy film than unmodified wool fat.

Reaction between wool fat and ethylene oxide produces a water soluble wax, melting at about 40°. It is a non-ionic surface-active agent which promotes the formation of oil-in-water emulsions and is also capable of acting as a solubilising agent. It could be useful in dermatological preparations since it combines some of the characteristics of wool fat with complete solubility in water.

Vegetable Oils and Related Substances

The vegetable oils have penetrating properties which are intermediate between those of the mineral oils and the animal fats (Harry, 1941). They are liable to become rancid on storage and this may be overcome by the inclusion of an antoxidant (Shotton, 1954). When used in dermatological vehicles, the possibility of these substances having skin sensitising properties must not be overlooked. Propyl gallate (0·05 to 0·15 per cent), tocopherol and butylated hydroxytoluene appear to be satisfactory but sensitivity reactions to hydroquinone have been reported (Lapin, 1942).

The acetoglycerides are a group of modified natural oils and fats obtained by substituting acetate groups in place of one or more of the fatty acid groups in the triglycerides (Newman, 1957). Any triglyceride may be modified by conversion to the mono- or diacetate and in the manufacturing process mixtures are obtained. Depending upon the nature of the raw materials and the conditions of the process, acetoglycerides are obtained ranging from mobile oily liquids to solid waxes.*

Acetoglycerides have a microcrystalline structure and produce thixotropic mixtures when blended with waxes and oils. Such mixtures retain their consistency over a wide range of temperatures. The acetoglycerides are stable to heat but can oxidise and commercial grades contain an antoxidant.

Skin penetration tests on the acetoglycerides have not been reported yet but their chemical nature suggests that they would penetrate the skin as readily as the natural oils and fats from which they are derived. They produce a film on the skin which is less greasy and occlusive than the mineral oils and wool fat.

Synthetic esters of fatty acids have also been used in the cosmetic industry to replace the natural vegetable oils. They have the advantages of more constant composition, lower acid values and they become less readily rancid than the natural oils. Isopropyl myristate (Hadgraft and Wolpert, 1960) is a clear, colourless, odourless liquid which is less viscous than arachis oil. In combination with the paraffins, it reduces their viscosity and increases the ease and rate with which they spread on the skin. Isopropyl palmitate and isopropyl palmitate-stearate are more viscous esters which give more "body" to the base.

* A. Boake Roberts & Co. Ltd.

The pharmacopoeial bases have been criticised by Howard (1960) who finds them unpleasant to use, coating the skin with a thick, greasy and occlusive residual film. He suggests that paraffin ointment could be improved if it were modified by the inclusion of acetoglyceride L/C or isopropyl myristate. A similar modification is suggested for simple ointment in which some of the soft paraffin should be replaced with isopropyl myristate. These modifications produce ointment bases which are pleasanter to use, less occlusive and do not interfere with the normal transport of water at the skin surface.

Emulsified Bases

Emulsified bases may be either water-in-oil emulsions (oily creams) or oil-in-water emulsions (aqueous creams). They are cosmetically superior to the older fatty type of base and are less likely to interfere with normal skin functions. They have a cooling effect from the evaporation of the water which they contain and are miscible with exudates, thus permitting drainage. Many emulsified bases contain surface-active agents which assist in bringing the active medicament into intimate contact with the skin surface.

Emulsification of an oil does not appear to enhance its ability to penetrate the skin (Harry, 1941). When a cream is applied to the skin, the aqueous phase evaporates leaving a film of oil, the penetration of which depends upon the nature of the oil phase and does not differ significantly from that obtained when the oil is applied alone. Creams containing mineral oils produce films which remain on the skin surface whereas those containing vegetable oils or wool fat produce films which slowly penetrate the skin. It should be noted that the concentration of active medicaments is greater in the residual film than in the original preparation. This needs to be taken into consideration in determining the strength and may possibly explain the greater efficiency of emulsified as opposed to oily preparations.

The rate of release of medicaments from emulsified bases is affected by the particular phase in which they are soluble. Oil-soluble medicaments incorporated into oily creams are dissolved in the external phase and come into immediate contact with the skin surface. Water-soluble medicaments are dissolved in the internal aqueous phase and are less immediately released. The release of drugs from aqueous creams follows a reverse order. Water-soluble substances are more immediately released from aqueous creams than from oily creams.

Oily Creams

The only official base of this type is oily cream which was discussed at the Symposium Session of this Conference in 1947 (Hadgraft). Its poor stability, particularly when combined with a number of commonly used medicaments, leaves a need which at present is not completely filled by an official preparation. The stability of oily cream has been shown by Clark and Kitchen (1960b) to be affected by the amount of autoxidation in the wool alcohols used in making the cream and by the method of

preparation. These authors have found that autoxidation is greatly reduced if the wool alcohols is stored as a mixture with liquid paraffin. Greater stability may also be obtained if Liquid Lanolin "60" is used as an auxiliary emulsifier.

Dr. Jarrett has referred to the possible role of zinc in keratin formation and it may be for this reason that zinc cream is frequently used as the vehicle for other dermatological medicaments. It is often combined with ichthammol with which it shows only poor stability. For this reason, a higher proportion of wool fat is included in zinc and ichthammol cream but even with this formulation, separation may occur on storage.

The fatty acid esters of the anhydrides of polyhydric alcohols may be of value in the formulation of stable oily creams. Many such substances are commercially available but they consist of mixtures which are not readily standardised and none are yet described in standard publications. Sorbitan mono-oleate and sorbitan sesqui-oleate are typical representatives which are soluble in vegetable and mineral oils and dispersible in water. They are free from irritant properties when applied to the skin (Dodd, Hartman and Ward, 1946) and can be combined with soft paraffin and wool fat to form oily creams which are stable in the presence of resorcinol, sulphur, ammoniated mercury, solution of coal tar and salicylic and benzoic acids.

Aqueous Creams

Surface-active agents for the formulation of aqueous creams can be divided into three main groups: (i) anionic, (ii) cationic and (iii) non-ionic compounds. A fourth group, the amphoteric surface-active agents (Moore, 1960) have not yet been used in dermatological preparations. For the production of stable emulsions, surface-active agents must be combined with an auxiliary material capable of forming a stable, close-packed, interfacial film (Schulman and Cockbain, 1940) and cetostearyl alcohol is commonly used for this purpose.

Anionic and cationic emulsifying agents such as self-emulsifying monostearin (Soulsby, 1940), emulsifying wax (Gunn, 1960; Hadgraft, 1947; Silcock and Chamings, 1939; Soulsby, 1940) and cetrimide emulsifying wax (Gunn, 1960) are incompatible with electrolytes in high concentration and with organic ions bearing a charge of opposite sign to the surface-active compound because neutralisation of the charged oil droplets interferes with the stability of the emulsion and the activity of the medicament may be reduced.

Non-ionic emulsifying agents possess both hydrophilic and lipophilic groups. The hydrophilic group is usually an ethylene oxide chain which can be combined with numerous lipophilic radicals. Compounds of this type may be polyethylene glycol ethers of fatty alcohols (Hadgraft, 1954) such as cetomacrogol 1000 or esters of polyethylene glycol and a fatty acid such as the macrogol stearates (Johnson and Thomas, 1955). They are incompatible with phenolic compounds with which they probably form compounds by hydrogen bonding (Mulley and Metcalf, 1956). Although non-ionic they possess a residual negative charge and may

inactivate cationic medicaments such as the quaternary ammonium compounds.

A third type of non-ionic emulsifying agent consists of the polyethyleneglycol derivatives of the esters of fatty acids and the anhydrides of polyhydric alcohols. In the formulation of aqueous creams, a mixture of sorbitan monostearate and sorbitan monostearate polyoxyethylene derivative is used. By varying the proportions of oil-soluble and water-soluble derivatives in mixtures of this type a range of emulsifying agents of varying hydrophile-lipophile balance may be obtained. To facilitate the selection of an emulsifying agent for a particular purpose, an empirical number (HLB value) has been ascribed to compounds of this type representing their hydrophile-lipophile balance (Griffin, 1949). Maximal stability is achieved at a specific HLB value characteristic of the oil phase concerned and the HLB values for emulsifying a range of commonly used oils and waxes may be obtained by reference to tables which have been compiled by Griffin.

More recently a linear relation between HLB values and spreading coefficients has been established (Becher, 1960). For stability in an oil-in-water emulsion, a negative spreading coefficient between the oil phase and an aqueous solution of the emulsifying agent is necessary to prevent oil globules from rising in the emulsion and spreading on the surface to form a separate phase. This is limited by the requirement that a low interfacial tension is also a necessary condition for emulsion stability. The emulsifying agent selected, therefore, must provide the most negative spreading coefficient consistent with a low interfacial tension. This can be determined by a simple practical method in which the oil phase is dropped on to a series of dishes containing solutions of emulsifiers of varying HLB values. The emulsifying agent having the highest HLB value which permits no spreading of the oil phase is selected. This method might profitably be extended to the investigation of incompatibilities encountered in the formulation of dermatological creams. For example, the incorporation of water-soluble substances such as ichthammol often produces creams of poor stability and the selection of an emulsifying agent which provides the most negative spreading coefficient for the oil phase in the presence of the medicament might indicate a more stable formulation.

Preservation of Aqueous Creams

Aqueous creams often support the growth of moulds and bacteria and a preservative may be required to prevent contamination during storage. Creams containing anionic or cationic emulsifying agents may be preserved with chlorocresol or the esters of hydroxybenzoic acid. Aqueous creams containing the non-ionic emulsifying agents present greater difficulties.

Much evidence indicates that non-ionic surface-active agents are capable of interaction with preservatives thus reducing their activity (Beckett and Robinson, 1958; Bolle and Mirimanoff, 1950; Navarre, 1957). However, the performance of methyl and propyl hydroxybenzoates in aqueous creams is sometimes better than is indicated by their activity

in the presence of a non-ionic compound in a liquid culture medium (Charles and Carter, 1959). Alternative preservatives which have been suggested are sorbic acid and a combination of sorbic acid and hexylene glycol (Barr and Tice, 1957).

A synergistic effect has been found to exist between the hydroxybenzoates and polyhydric alcohols such as propylene glycol, 1,3-propanediol and 1,4-butanediol (Poprzan and Navarre, 1959). It has been suggested that this arises from the interference with the compound formed between the preservative and the non-ionic agent. Aqueous creams containing non-ionic emulsifying agents may be preserved with the hydroxybenzoates, providing the formulation contains at least 10 per cent of propylene glycol.

More recently, the view has been advanced (Hibbott and Monks, 1960) that the performance of methyl hydroxybenzoate in aqueous creams depends upon its partition between the oil and the aqueous phase and to be effective, it must be present in the aqueous phase in adequate concentration. This is consistent with the finding that methyl hydroxybenzoate is more effective in a cream containing mineral oil, in which it is poorly soluble, than in one containing isopropyl myristate, in which the ester is appreciably soluble. When non-ionic emulsifying agents are present, the ester is partitioned not only between the oil and the aqueous phase but its effective concentration is further reduced owing to its solubility in the emulsifying agent. The addition of propylene glycol and similar substances increases the solubility of the preservative in the aqueous phase and this may account for its greater efficiency in such formulations.

Water-Soluble Ointments

The macrogols, two of which have been included in the B.P.C., are simple polyethylene glycols which are used in the formulation of water-soluble ointment bases. The higher members of the series such as hard macrogol are unique substances in combining complete solubility in water with a wax-like consistency. A number of authors have reported on their use in the formulation of ointments (Hopkins, 1946; Landon and Zopf, 1943; McClelland and Bateman, 1949; Meyers Nadkarni and Zopf, 1950) and they have been shown to be relatively free from skin sensitising properties. However, two instances of demonstrable sensitivity to the polyethylene glycols have been reported (Strauss, 1950).

There is some evidence that medicaments may be more readily absorbed from macrogol bases than from paraffin bases (Meyers, Nadkarni and Zopf, 1949) and it may be necessary to reduce the concentration if macrogol ointment is substituted for a paraffin base. Moreover, it has also been reported that the macrogols may increase the possibility of sensitivity reactions of diseased skin to the active medicament in ointments (Sulzberger and Baer, 1953). Ointment bases containing the macrogols are hygroscopic and the surface may become moist on storage. They have only a limited use in the formulation of dermatological preparations but may be of particular value when it is desired to wash the preparations from the skin.

Pressurised Aerosols

Pressurised containers have recently been used for the application of medicaments to the skin. The formulation of such preparations is outside the scope of this review and has been the subject of a number of articles (Briston, 1958; P.A.S.R., 1957; Root, 1956; Sciarra, Tinney and Feely, 1960; Streatfield, 1955). Only those aspects which affect the performance of the preparation on the skin will be discussed here.

A propellant is used which is usually a liquified gas of the fluorinated hydrocarbon series or a mixture of these. The most commonly used propellants are Arcton 11 (trichlorofluoromethane), Arcton 12 (dichlorodifluoromethane) and Arcton 114 (dichlorotetrafluoroethane). Carbon dioxide and nitrogen may also be used. In one kind of formulation the medicament is dissolved in a solvent which may be miscible or immiscible with the propellant. For this purpose, ethanol, isopropanol, propylene glycol and the liquid macrogols have been used. The solution is discharged from the container in the form of a fine mist and the propellant is assumed to be completely vaporised before the preparation reaches the skin. Even so, the possibility of sensitivity reactions to the propellant needs to be considered. A more serious possibility is the risk of reactions to the solvent in which the medicament is dissolved. There have been reports of a burning sensation from isopropanol (Thorne, 1959) and of irritation from propylene glycol (*Prescribers J.*, 1961). These are hazards which apply to the same substances when used in any other form of topical application.

In another kind of formulation, the medicament is dispersed in the propellant and is delivered to the skin surface as a fine powder. Such preparations may be of particular value in the application of antibiotics to infected areas.

Emulsified preparations may also be dispensed in pressurised containers but at present no dermatological preparations of this kind are available. The possibility of some propellant remaining in the preparation after application to the skin and a consequent increased risk of sensitivity reactions would need to be carefully considered. Carbon dioxide or nitrogen may be used as alternative propellants but have the disadvantage that the pressure falls as the container is emptied. About 10 per cent of the product may not be discharged and it would be a wasteful method for dispensing an expensive medicament. Creams which are passed through pressurised containers acquire foam characteristics since they are aerated by the propellant and homogenised by the valve. Consequently, they possess very good cosmetic properties.

Aerosol formulations have a number of advantages for skin medication. The preparation may be kept sterile and out of contact with air until the time of use. The medicament is applied directly to the skin surface by a no-touch technique and this may be of particular value in the treatment of conditions which are susceptible to secondary infection. The efficiency of distribution may also be important in covering a large area of the skin with the minimal amount of medicament in a finely divided form. But doubt has been expressed whether significant amounts of hydrocortisone

are absorbed when applied by spraying (*Prescribers J.*, 1961) and the precise value of such formulations must await further clinical assessment.

Skin Penetration and Absorption

In the treatment of skin diseases, it is seldom necessary for the medicament to be absorbed percutaneously, but penetration of the preparation to the deeper layers may be necessary. In such circumstances, the possibility of systemic absorption may be an undesired hazard (Snyder, 1960).

Percutaneous absorption has been the subject of a number of reviews (Blank, 1960; Gemmell and Morrison, 1957; Griesemer, 1960; Hadgraft and Somers, 1956). The main route by which substances pass through the skin is by the hair follicles, although autoradiographic studies have recently demonstrated that the corticosteroids are capable of true transepidermal penetration. The sweat glands may also play a part in the absorption of medicaments through the skin.

In general, lipid-soluble substances penetrate the skin more readily than water-soluble substances. If lipid-solubility is combined with water-solubility, the substance is likely to be absorbed at the base of the hair follicle. The vehicle in which the medicament is formulated may have a modifying influence on the amount absorbed (Gemmell and Morrison, 1958, a, b). The most important factor concerned is the physicochemical nature of the drug and it is doubtful whether pharmaceutical formulation is capable of promoting the absorption of a substance which is not alone capable of being absorbed. Some substances are very readily absorbed by the skin and occasionally this may form a useful route for the administration of drugs. Ditophal, which is used in the treatment of leprosy, is rapidly and completely absorbed by the skin and releases ethyl-mercaptan in the tissues.

The corticosteroids are extensively used in topical preparations and the possibilities of percutaneous absorption are of particular importance in this group of substances. Hydrocortisone is absorbed percutaneously through both the transepidermal and pilosebaceous routes. The amount absorbed is small and is about 1 per cent of the total dose applied to normal skin. Absorption from inflammatory sites is greater and may be as high as 15 per cent of the dose applied to the skin. Fludocortisone is also absorbed percutaneously and since its systemic effects are produced by small doses, it is the only analogue of cortisone known to be capable of eliciting systemic effects after the use of topical preparations (Malkinson, [sic]1960).

Conclusion

Preparations formulated for skin medication should be kept as simple as possible. Water-soluble medicaments are probably best applied to the skin in aqueous solution. Ease of application and localisation of effect may be achieved either by the addition of hydrophilic colloids such as sodium carboxymethylcellulose or by emulsification with an oil to form an aqueous cream. The nature of the oil phase will depend upon the condition under treatment but the materials added to assist pharmaceutical

formulation should be kept to a minimum to avoid adding materials which are likely to interfere with the activity of the medicament or to produce sensitivity reactions. The chosen emulsifying agent will depend upon the active medicament but there are now available anionic, cationic and non-ionic emulsifying agents which permit the formulation of creams suitable for all kinds of medicaments.

Oil-soluble medicaments are best applied in oily creams but at the present there is no entirely satisfactory official preparation for this purpose. Oil-soluble medicaments may also be incorporated in anhydrous bases.

The water-soluble bases such as macrogol ointment have only a limited use since they do not appear to be free from irritant effects.

The effects of pharmaceutical formulation on the activity of topically applied medicaments has not been fully assessed clinically. Probably, the most that can be achieved is to present the medicament in a form which enables it to exert its maximal activity in a preparation which is cosmetically as acceptable as possible. For this purpose, additional materials which are in use in the cosmetic industry are applicable to the formulation of dermatological preparations.

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Discussion

The following points arose out of the discussion.

Despite newer techniques and materials, the formulation of skin medicaments relied much on empiricism. Laboratory tests, such as the FDA cup plate method, could give useful information about the release of medicaments from bases. Although animal studies helped in assessing absorption and penetration and the sensitising and irritant properties of bases and medicaments, it was not easy to draw firm conclusions from them. Radioactive materials were valuable in the study of how absorption into the skin was affected by dermatological conditions but the techniques were very sensitive and there was a danger of over-optimistic interpretation of the results. Although propylene glycol had been found to be a sensitising agent, this was not the experience of all dermatologists; selenium sulphide was similar in this respect. Psychological disturbances could make an established skin disease worse but were not usually the causative agents: tranquilising drugs were of limited value in dermatology. Goosegrease still compared favourably with modern bases. Many modern

medicaments were available in both greasy and non-greasy bases. Since greasy bases encouraged hydration of the keratin layer, there were grounds for using this type of base. Some substances could penetrate the skin by different routes: it was necessary to distinguish between trans-epidermal penetration and passage through the hair follicles and glands. The azo dyes were now seldom used in dermatology.

CONFERENCE LECTURE

INTERFERON: A ROUND UNVARNISH'D TALE

By Alick Isaacs, M.D.
National Institute for Medical Research, Mill Hill, London

It is a great honour to be asked to lecture to the British Pharmaceutical Conference and a particular honour to be invited to give the first of a series of annual lectures. Faced with the difficulty of finding words of my own in which to thank you adequately I have taken refuge in Shakespeare:

> "Yet by your gratious patience
> I will a round unvarnish'd tale deliver . . .
> . . . what drugs, what charms,
> What conjuration and what mighty magic,
> For such proceeding I am charg'd withal."

Research on interferon represents an exciting example of how investigation in what appears to be a wholly academic field of research can lead to more practical prospects. In the laboratory it has been known certainly for 25 years that when cells are infected with one virus they acquire resistance to infection with other unrelated viruses. This is the phenomenon known as virus interference. Scientists were curious about the nature of this cellular resistance and indeed virus interference encouraged research from workers connected with the viruses of plant, bacterial and animal cells. Once the phenomenon had been defined, an important step forward came when it was demonstrated first with bacterial (Delbruck and Luria, 1942) and later with animal viruses (Henle and Henle, 1943) that a virus which had been killed by treatments such as heat or ultra-violet irradiation could lose the power of multiplying in cells but retain the ability to interfere with the growth of other viruses. This gave an opportunity of clarifying the problem. One could then pose questions such as: does the killed virus block the entry of further viruses into the cell, and does the killed virus block an early stage or a late stage in the virus multiplication cycle? This and related topics were investigated in a number of laboratories and it was shown that the killed virus did not prevent the entry of living virus and also that the interference probably occurred at an early stage of the virus growth cycle, that is to say, not only was virus formation prevented but the formation of the building blocks that go to make up the mature virus particles was also inhibited.

The next stage in work on viral interference came in 1957 with the discovery of interferon (Isaacs and Lindenmann, 1957). It was shown that when cells were treated with killed influenza virus a viral inhibitory substance was produced which could be separated from the killed virus, isolated and shown to confer resistance on fresh cells. Essentially this

work involved at first an explanation of the phenomenon of virus interference; we were not consciously searching for an antiviral substance and this only appeared as a later development in the work. At an early stage of this work we made a guess that interferon production represented an abortive attempt of the cell to synthesise virus, but with further investigation it became clear that interferon was completely different from the virus used to induce its production. We now think of interferon rather as a response of the cells to the stimulus of virus infection.

The next question is: what kind of cells respond by producing interferon and what kinds of virus can be used to initiate this response? At the moment we don't know whether viral interference in plant and in bacterial viruses can be explained by the production of a substance similar to interferon. All we can say is that this seems to be rather a general response of those vertebrate cells that have been investigated. Cells of chickens, ducks, mice, hamsters, rabbits, ferrets, cattle, pigs, dogs, monkeys and man have been shown to produce interferon and to be sensitive to its antiviral action.

So far all the animal viruses that we have tested have been shown to initiate the production of interferon, although the actual amount induced by different viruses varies considerably. The viruses tested include small viruses such as poliomyelitis and encephalitis, medium sized viruses such as influenza and mumps, and large viruses such as those of the pox group. Tumour viruses also induce production of interferon. Viruses can be used both live and inactivated, but since any preparation of live virus is not homogeneous and since multiplication can be initiated by only a small proportion of particles in the virus population it is difficult to say which particles are responsible for initiating interferon production. It seems unlikely from what is known at present that interferon is produced by cells at the same time as they are actively engaged in synthesizing virus. It is more likely that the cell can respond to infection in one of two ways—by producing interferon, which then gives it protection against virus multiplication, or alternatively by synthesizing virus. It should be important to try to understand which factors govern the particular pathway that the cell will follow on infection with a virus particle.

It looks, therefore, as if interferon production can be thought of as a cellular response to infection. An early finding in this work was that interferon is liberated spontaneously from cells. It therefore has the possibility of entering the surrounding cells and protecting them. In other words, interferon may not only protect the cell that produces it but the organism itself. This raises the question of whether interferon plays some role in our defences against virus infection. We can consider resistance against virus infection under two headings, firstly recovery from a first infection, and secondly prevention of re-infection. The success of virus vaccines is compelling evidence of the importance of antibody in our ability to resist re-infections. What is not so clear is whether antibody plays anything like such an important role in our ability to recover from primary infections. Doubts about the importance of antibody spring from the fact that recovery from many virus infections

seems to occur at a time before antibody is particularly evident. Also, the experimentalist in the laboratory is continually faced with examples of cells recovering from virus infection *in vitro* under conditions where antibody production does not occur. It is clear, therefore, that other mechanisms must play a role in the recovery processes. It was natural to wonder whether interferon could be important in this respect and careful investigation of tissue cultures chronically infected with a number of different viruses has shown that it is possible for virus and cells to learn to live together in peace over long periods of time. The resistance of these chronically infected cells appears to be due to production of interferon in the cultures (Ho and Enders, 1959; Henle, Henle, Deinhardt, and Bergs, 1959). The same problem can be studied in chick embryos which have been found to show varying resistance to viral infection at different ages. Chick embryos of less than 7 days old show a much lower survival rate after infection with many different viruses than do chick embryos of more than 8 days old. The time at which change in susceptibility occurs appears to be critical and it is closely correlated with the time at which the tissues of the growing embryo develop sensitivity to the antiviral action of interferon (Baron and Isaacs, 1961). These findings suggest that the ability of chick embryos to survive virus infections is closely linked to their ability to produce interferon, to which their cells are sensitive.

Two substances have been found to inhibit the antiviral action of interferon. They are oxygen at high concentrations (Isaacs, Porterfield and Baron, 1961) and cortisone (Kilbourne, Smart and Pokorny, 1961). It was striking to find that increased oxygen tension and cortisone both have a detrimental effect on the course of virus infections. In the case of increased oxygen tension this was shown by experimental infection of mice with influenza virus. Animals kept under increased oxygen tension died more rapidly than animals kept in air (Sawicki, Baron, and Isaacs, 1961). In the case of cortisone this is a clinical observation which has been known for the last few years. It is recognised that patients under treatment with cortisone are at special risk from infection with chicken pox virus. These findings again support the idea that interferon plays an important role in recovery from virus infections.

Other factors too are clearly involved in recovery from virus infection. It is known that high tempertaures inhibit the growth of a number of viruses and Lwoff (1959) has suggested that fever may play an important role in helping in recovery from virus infections. Again, many viruses do not develop well at a low pH, and an inflammatory exudate of low pH may therefore help to play a defensive role. One wonders if these different mechanisms are unconnected or if they may all be linked together. It is possible that raised temperature and lowered pH may act by stimulating the production or the action of interferon. These suggestions will soon be tested experimentally.

Interferon is a protein of molecular weight 63,000 (Burke, 1961) and it acts by protecting cells against virus infection. It has no direct action on virus outside the cells. We have recently found that interferon can

be added to cells in high concentration without inhibiting to any great extent the growth and multiplication of the cells. Such treated cells are, however, highly resistant to virus growth. This suggests that interferon must act by inhibiting the synthesis of viral nucleic acid or protein without significantly inhibiting the production of nucleic acid or protein required in the economy of normal cells. The mode of action of interferon must therefore be quite a subtle one. It is possible that the production of "foreign" nucleic acids or "foreign" proteins is subjected to different control mechanisms from those that control the production of normal cell nucleic acids and proteins and that interferon can block the former without affecting the latter. There is indirect evidence that interferon may act on an oxidative mechanism which is required for producing energy for viral synthesis. At the moment, however, there is no direct evidence on this point, nor is it clear how an oxidative mechanism can be more required in the production of a foreign nucleic acid or protein than in the production of normal cell nucleic acid or protein. This subject will therefore require much further investigation.

Another field which will require investigation is the function of interferon in the normal economy of the body. It seems unlikely that a general property of cells, that is, their ability to produce interferon when stimulated with a large variety of different viruses, should have developed in the course of evolution solely as an antiviral defence mechanism. It seems much more likely that interferon plays another role in the normal economy of the body but that relatively recently in evolution this mechanism should have become adapted to deal with viral infections. One would imagine, therefore, that interferon might play a role in normal cells in controlling the synthesis of nucleic acid or protein of an unusual kind. In speculating about this process one is struck by the fact that interferon shows a very weak antiviral action in the cells of very young embryos and in cancer cells. The implication is that this hypothetical synthesis of a "foreign" nucleic acid or protein may play a role in the processes concerned with differentiation in the very young embryo and that this does not occur in the fully differentiated cells of the normal adult; cancer cells might then be considered as cells which have escaped from this controlling mechanism. These speculations are supported by very little evidence but they have the merit of suggesting a number of experiments.

Interferon has clearly many theoretical points of interest surrounding its mode of action but it has too a practical interest. Laboratory experiments show that it has an antiviral action which extends to a very wide range of animal viruses and that it can be given to cells in very large doses without apparently causing any significant toxic effect. It does seem too that if interferon plays a normal role in recovery from virus infections that the attempt to use it as an antiviral agent in man would be a logical attempt to improve on a natural mechanism of recovery. This is the reasoning which has prompted the Medical Research Council to set up a collaboration with three pharmaceutical firms and it is hoped that in the near future this collaboration will have reached the stage when it should be possible to test out interferon in man. Experiments in animals have

been encouraging and we are hopeful that preliminary experiments may at least repeat a pattern found in experiments in animals. But even if this works out well it is only a first stage in a long investigation required to improve the yields of interferon over those obtainable at the moment, to learn if possible to increase its antiviral effect and to learn how best to use it in the treatment of virus infections in man. The success of such a venture depends on our ability to collaborate in an interesting investigation and one hopes that this partnership between the Medical Research Council and the British pharmaceutical industry will be extended in the future to many other fields of investigation.

We began this work with the investigation of an odd phenomenon, viral interference, and the research has taken us on an interesting journey. Some parts of the road are quite well mapped out already, while for others we will have to retrace our steps and investigate the pathway once more. Yet the glimpses we have of the road ahead make us eager to press on with the journey. The destination is the understanding of what interferon is and how it acts. The closer we can reach to this destination the more rationally shall we be able to use interferon in the treatment of virus infections of man.

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SCIENCE PAPERS

THE STABILITY OF SOLUTIONS OF THE INTERNATIONAL PYROGEN REFERENCE PREPARATION

By C. H. R. Palmer and T. D. Whittet

From the School of Pharmacy, Leicester College of Art and Technology, Leicester, and the Pharmaceutical Department, University College Hospital and Medical School, London

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The stability of dilute solutions of the International Pyrogen Reference Preparation has been studied. When the response is plotted against log dose a straight line is obtained. Steaming for 30 min. causes a loss of at least 52 per cent of activity and autoclaving at 115° for 30 min. practically complete destruction. Storage in a refrigerator (approximately 4°) for 16 weeks caused a loss of activity. The pyrogen is removed from solution by a strongly basic anion exchange resin and is unaffected by a strongly acidic cation exchange resin.

The desirability of a standard for pyrogens was first discussed at a meeting of the World Health Organisation Expert Committee on Biological Standardisation (W.H.O., 1951), when the problem was outlined by Paton (1950). After a further meeting, held in 1953 (W.H.O., 1953), two pyrogens were made available for study—a partially purified extract from *Proteus vulgaris* and a purified lipopolysaccharide from *Serratia marcescens*. The results of these studies were inconclusive and, at a meeting held in 1956, a highly purified pyrogen from *Shigella dysenteriae* was chosen as a purer and more easily reproducible product (W.H.O., 1959).

The preparation and purification of this material were described by Davies, Morgan and Mosiman (1954) and its properties and method of handling by Humphries and Bangham (1959). Its full description is the "O" somatic antigen of *S. dysenteriae* (shiga) (type I of Kaufmann, 1951), strain K.624 "smooth".

Todd (1955) recommended the adoption of Westphal's lipopolysaccharide from *Salmonella abortus equi* ("Pyrexal") as a standard and has used it as such. He has criticised the choice of the *S. dysenteriae* pyrogen (Todd, 1960).

Since it is obviously desirable to compile as much information as possible on the new reference preparation several studies were undertaken.

Experimental

The International Pyrogen Reference Preparation (IPRP) was obtained as a freeze-dried solid in ampoules each containing 2 mg. A solution was made in water for injection and this was distributed into sterile ampoules to give quantities of 0·25 mg. of IPRP. The solution was then freeze-dried and the ampoules were sealed.

When required, dilutions containing 50 ng. IPRP per ml. in water for injection were prepared from these ampoules on the day of experiment.

An intravenous dose of 1 ml./kg. of this dilution is sufficient to produce a rise of temperature of about 1° in a rabbit.

The animals used were male rabbits of several breeds, weighing between 2 and 4 kg. They had all been injected previously with pyrogens but were not tolerant. They were fed on the National Institute for Medical Research Diet No. 18 with cabbage and drinking water in addition. Food and drink were withheld during tests. Rectal temperatures were measured with a thermistor apparatus (Whittet, 1958). All injections were given into ear veins, in the early afternoon at approximately the same time in each experiment.

TABLE I

EFFECT OF VARIOUS DOSES OF INTERNATIONAL PYROGEN REFERENCE PREPARATION ON THE TEMPERATURES OF RABBITS

Dose ng./kg.	No. of tests	Mean response °	S.D.
50·0	12	1·05	±0·30
10·0	12	0·62	±0·33
2·0	12	0·28	±0·17
0·4	12	0·12	±0·07

TABLE II

EFFECT OF HEAT ON THE STABILITY OF THE INTERNATIONAL PYROGEN REFERENCE PREPARATION

Dose 50 ng./kg.	No. of tests	Mean response °	S.D.
Unheated solution	12	1·02	±0·34
Steamed solution	12	0·49	±0·30
Autoclaved solution	12	0·23	±0·15

TABLE III

EFFECT OF STORAGE ON THE STABILITY OF THE INTERNATIONAL PYROGEN REFERENCE PREPARATION

Dose 50 ng./kg.	No. of tests	Mean response °	S.D.
Fresh solution	12	1·08	0·32
Solution stored 9 weeks at 4°	3	1·16	—
Solution stored 16 weeks at 4°	3	0·58	—

Construction of log-dose-response line. Twelve rabbits and four doses (50, 10, 2 and 0·4 ng./kg. weight) were used.

Three rabbits were each given a dose of each level on four different occasions until each animal had received each dose. The order of administration was decided by means of a Latin square.

The mean responses of 12 rabbits to each dose, together with their standard deviations are shown in Table I.

Effect of heat. Solutions containing 50 ng./ml. of IPRP were treated (*a*) by steaming at 90 to 100° for 30 min., (*b*) by autoclaving at 115° for 30 min. When cool the solutions were injected into rabbits in a dose of 1 ml./kg., unheated samples being used as controls. The results are shown in Table II.

Effect of storage. A solution of IPRP (50 ng./ml.) was tested when freshly made and again after storage at approximately 4° for 9 weeks and

then for 16 weeks. A dose of 50 ng./kg. was used. The results are shown in Table III.

Effect of ion-exchange resins. Solutions containing 50 ng./ml. of IPRP were passed through columns (approx. 15 cm. in length and 3 cm. in diameter) of either Zeocarb 225 (cationic) in the hydrogen form or Deacidite FF (anionic) in the hydroxyl form. The effluents were injected into rabbits in a dose of 1 ml./kg. The results are shown in Table IV.

TABLE IV

EFFECT OF ION-EXCHANGE RESINS ON THE INTERNATIONAL PYROGEN REFERENCE PREPARATION

Dose 50 ng./kg.	No. of tests	Mean response °	S.D.
IPRP solution:			
Untreated	12	0·96	±0·31
Treated with Zeocarb (cation resin) ..	12	0·81	±0·30
Treated with Deacidite FF (anion resin)	12	0·30	±0·37

RESULTS AND DISCUSSION

The theoretical mean response value for each dose used in the construction of the log-dose response line was calculated from the experimental data by means of the equation $Y = \bar{y} + b(x - \bar{x})$. They were 50 ng./kg. = 0·99°; 10 ng./kg. = 0·68°; 2 ng./kg. = 0·36°; 0·4 ng./kg. = 0·07°.

The results show that the preparation gives a log-dose response line in which the practically determined points are close to the calculated best straight line. A dose as low as about 5 ng./kg. is sufficient to give a rise in temperature sufficient to fail the B.P. pyrogen test.

Comparison of the response of unheated IPRP solution with that of steamed solution showed that at least 52 per cent of the activity was destroyed.

The autoclaved solution gave a mean rise in temperature of only 0·30°. Assuming that this is all due to undestroyed pyrogen, autoclaving by the pharmacopoeial process has caused destruction of 70 per cent of the activity. In practice, however, a mean response of about 0·3° is not uncommon in a negative pyrogen test and the B.P. allows a mean rise of 0·33° in a test using 3 rabbits and of 0·55° in a test using 12 rabbits before a solution fails the test.

The mean response in 12 rabbits of the steamed solution is 0·49° and that of the autoclaved solution 0·30° and thus both would pass the B.P. pyrogen test.

Whittet (1961) found a mean response of 0·21° in 100 pyrogen tests on products made with every precaution to avoid pyrogens and believed to be pyrogen-free.

The responses of the 12 tests on autoclaved solution were compared with those of 12 tests on solutions believed to be pyrogen-free (mean response 0·20°) by means of the *t* test and were found not to be significantly different ($t = 0·0924$; $P = >0·9$; D.F. = 22). The difference in response between the unheated and autoclaved solutions was highly significant ($t = 7·999$; $P = <0·001$; D.F. = 22). The difference in response

between the unheated and steamed solutions was also highly significant ($t = 8{\cdot}135$; $P = <0{\cdot}001$; D.F. $= 22$).

In the storage tests no significant destruction had occurred in 9 weeks, but by 16 weeks almost half the activity had been lost.

The tests with ion-exchange resins showed that IPRP is probably completely adsorbed by the strongly basic anion exchange resin Deacidite FF in the hydroxyl form and is unaffected by the strongly acidic cation exchange resin Zeocarb 225 in the hydrogen form. The difference between the responses of untreated solution and that treated by Deacidite FF is highly significant ($t = 5{\cdot}013$; $P = <0{\cdot}001$; D.F. $= 22$). The difference between untreated and Zeocarb treated solution is not significant ($t = 0{\cdot}8810$; $P = 0{\cdot}4$ to $0{\cdot}3$; D.F. $= 22$).

With the exception of its reaction towards heat, the International Pyrogen Reference Preparation has the properties usually attributed to bacterial pyrogens, especially those from Gram-negative organisms. Thus, if the response is plotted against log-dose, a straight line is obtained. After intravenous injection of a suitable dose into rabbits a typical fever curve is obtained after the usual latent period of 20 to 30 min. With high doses a secondary rise in temperature occurs.

The effect of ion-exchange resins on IPRP resembles that on the pyrogens of *P. vulgaris*, *S. abortus equi* and *Pseudomonas aeruginosa* (Whittet, 1956, 1958).

Since the early reports by Seibert (1923) and those of Banks (1934), which dealt with only two pyrogens from named organisms (*Pseudomonas ureae* and *Pseudomonas scissa*), there has been little systematic work on the stability of pyrogens.

On the basis of their work, it appears to have been accepted that all pyrogens are extremely thermostable. Wylie and Todd (1948, 1949) examined the effect of autoclaving at 120° on pyrogens from *P. vulgaris*, *Bacillus subtilis*, *Ps. aeruginosa* and *Micrococcus tetragenes*. The first three lost 50 per cent of their activity in 30 min. and 95 per cent in 2 hr. *M. tetragenes* pyrogen, however, lost only 20 per cent of its activity in 2 hr. They concluded that the pharmacopoeial autoclaving process would have little effect on a dilute solution of pyrogen such as might arise from accidental contamination of water and that sterilisation is of no practical value in removing pyrogen from solutions intended for parenteral administration. Whittet (1958, 1961) showed that the pyrogenic activity of London tap water is completely destroyed by autoclaving at 115° for 30 min.

There is much variation in the stability of pyrogens from different sources and, now that numerous different purified preparations are available, further stability studies should be made.

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The paper was presented by DR. WHITTET.

THE REMOVAL OF AIR DURING AUTOCLAVE STERILISATION OF FABRICS USING LOW PRESSURE STEAM

By G. R. Wilkinson and F. G. Peacock

From the Research Division, Allen & Hanburys Limited, Ware

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A modified sterilising cycle has been evolved after studying the temperature distribution and effects of moisture in fabrics in the pre-sterilisation phase. A vacuum of 20 mm. Hg abs. followed by the introduction of steam to raise the pressure to about 200 mm. abs. and then re-applying a vacuum of 20 mm. Hg abs. was found to produce the required conditions to give even heating throughout the materials.

Removing air from the interstices of fabrics by downward displacement is a protracted and uncertain procedure. Evacuating to about 200 mm. Hg abs., introducing steam to above atmospheric pressure and re-evacuating to the same vacuum before introducing steam for the sterilising period removes more air but this procedure is unreliable (Savage, 1937; Perkins, 1956; Alder and Gillespie, 1957). Evacuating the chamber and dressings to below 20 mm. Hg abs. (B.S. 3220/1960) produces accepted sterilising conditions within the chamber but it is now doubted whether these conditions are always obtained within woven fabrics such as towels and gowns. The work upon which this cycle was based (Knox and Penikett, 1958) took no account of the moisture content of the dressings but the relation of moisture and superheating has since been discussed (Henry, 1959).

Previously the importance of the moisture content of dressings undergoing sterilisation by downward displacement has been commented upon (Barson, Peacock, Robins and Wilkinson, 1958) and a more critical examination of the high preliminary vacuum sterilising cycle has now been made. We report the influence of the moisture content and temperature of dressings on the reliability of sterilisation when high preliminary vacuum is employed and also a means of improving the reliability of dressing sterilisers.

Experimental and Results

Two autoclaves were used; autoclave A, and its recording apparatus, have been described (Barson, Peacock, Robins and Wilkinson, 1958). Autoclave B was jacketed, rectangular, 92 × 66 × 132 cm. deep, built to B.S. 1500/1960 and fitted with automatic control to B.S. 3220/1960 as well as manual control. Modifications were made to introduce steam for a period during stage 1 of the automatic cycle and for the cycle to be continued after the normal automatically controlled period had elapsed.

We chose 135° (equivalent to steam at 2·11 kg./cm.^{2} gauge) as the experimental temperature, which would need a sterilisation time of 3 min. (M.R.C. Working Party Report, 1960). Thirty huckaback towels, 56 × 76 cm., were folded and packed into a dressing casket, 28 × 28 × 25

cm. deep. so that the folds were in a vertical plane. They were allowed to equilibrate with atmospheric conditions. In this condition they contained 6 to 7 per cent of moisture. The dressing drum was positioned in the centre of autoclave A with a thermocouple near the geometrical centre of the pack. The autoclave was evacuated to 20 mm. Hg abs., steam introduced to 2·11 kg./cm.^{2} gauge (30 p.s.i.g.) for a pre-determined period. In 30 replicate experiments where the temperature at the centre of the pack was compared with the drain temperature, the centre of the pack took between 0 and 15 min. to attain the same temperature as the drain (Fig. 1). In a subsequent 20 experiments, 3 thermocouples were arranged vertically separated from one another by about 2 cm. in the centre of the pack. In all experiments some thermocouples were slow to

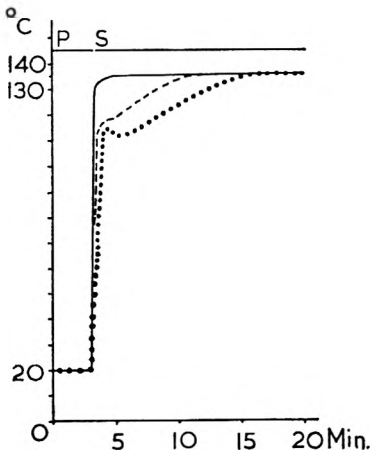

FIG. 1. Normal BS.3220/1960 Cycle (manually extended).

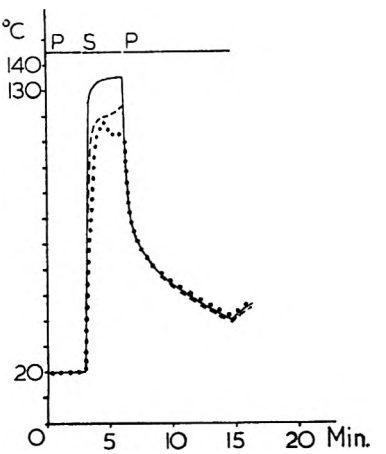

FIG. 2. Normal BS.3220/1960 Cycle (automatic).

Key to Figs. 1, 2, 3 and 4. —— Chamber and upper position in casket. – – – Median position in casket. ····· Lower position in casket. P—Pumping period. S—Steaming period.

reach chamber temperature and it was impossible to predict which position would be the slowest. With 5 thermocouples similarly disposed the effect was more noticeable and the random distribution confirmed in 20 replicate experiments.

In autoclave B, using manual control, the experiments were repeated more than 30 times and the random distribution confirmed. With automatic control (B.S. 3220/1960) some thermocouples did not attain the experimental temperature (Fig. 2) and spore papers positioned close to these thermocouples were not sterilised. Similar results were obtained in experiments using ten replicates when the packs were initially heated to 40° and 50°.

The moisture content of packs was adjusted by adding water to fabrics previously dried to constant weight at 100 to 105°, allowing them to equilibrate in a closed container and re-weighing immediately before positioning in the autoclave. The moisture content was calculated as

a percentage of dry weight. Replicate series of experiments were made using fabrics containing less than 5 per cent, 11 to 12 per cent, 20 per cent and 40 per cent of moisture and the results obtained were similar to those using air dried fabrics.

Further experiments were made using only 20 towels within the casket, but although the temperature range within the casket was reduced a distribution still existed.

The temperature distribution was smallest in experiments where the temperature of the fabrics fell during the preliminary vacuum stage. To examine this effect further, pumping was continued for up to 20 min. after 20 mm. Hg abs. pressure had been reached, during which time the pressure fell to about 6 mm. Hg abs. and the temperature of the fabrics

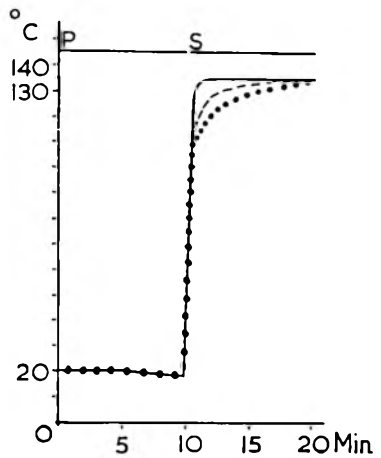

FIG. 3. Cycle using extended preliminary vacuum. See Fig. 1 for key.

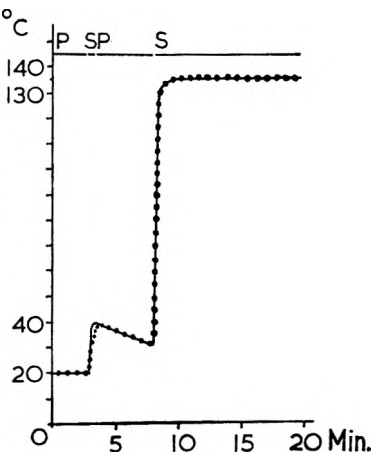

FIG. 4. Cycle with steam injection during the preliminary vacuum stage. See Fig. 1 for key.

dropped to about 19°. When steam was introduced the temperatures reached within the pack after the sterilising time of 3 min. showed less range, but were not uniform. Fig. 3, using 10 min. pumping is typical of the results for the series.

In further experiments steam was introduced into the chamber after the first vacuum, followed by re-evacuation to 20 mm. Hg abs. During this period the temperature of the pack fell and when steam was introduced for the sterilising period all thermocouples reached chamber temperature together.

The amount of steam admitted after the first evacuation was varied by increments in successive experiments so that the pressure within the chamber was increased from 20 mm. Hg. abs. to various pressure levels between 50 mm. Hg abs. and 2·11 kg./cm.^{2} gauge. It was found that an increase in pressure by means of steam to about 200 mm. abs. increased the temperature of the fabrics to 40–45° and subsequent evacuation to about 20 mm. Hg abs. cooled them to 35–40°. When steam was admitted

for the sterilising period, sterilising conditions were recorded by all thermocouples (Fig. 4). This behaviour was proved to be independent of the load or its initial moisture content. Similar experiments were conducted using autoclave B filled to capacity with fabrics in caskets and dressing drums, and repeated satisfactory time-temperature conditions suitable for sterilising were achieved in all parts of the load. This was confirmed by the use of *B. stearothermophilus* spore papers distributed throughout the load.

To challenge the method still further the slides over the apertures of the caskets were closed before being submitted to the cycle and the drums were laid so that the layers of fabric were horizontal. Thermocouples were also positioned in folded rubber sheets within these drums. In all cases the experimental temperature was reached consistently and confirmation of sterilising conditions was obtained by the use of spore papers.

The automatic cycle for autoclave B was modified so that the sequence became: evacuate to 20 mm. Hg abs., admit steam to 200 mm. Hg abs., evacuate to 20 mm. Hg abs., steam to 2·11 kg./cm.^{2} gauge for 3 min., evacuate to 40 mm. Hg abs., admit air. During the second evacuation the temperature of the fabrics fell from about 45° to under 40°, although the jacket was maintained at 135°. During the second steam period all thermocouples within the pack recorded temperatures which were contiguous with that recorded by a thermocouple in the chamber drain. Numerous replicate experiments produced similar results.

Discussion

Our experiments show that the upper portion of the pack frequently reached the same temperature as the chamber drain, while the centre portion about one-quarter from the base of the pack was usually at a lower temperature. The delay of a part in reaching chamber temperature is probably due to retained air being displaced downwards through it from the interstices above. This effect does not occur in the uppermost region because there is only steam in it. The residual air is compressed towards the centre by the incoming steam and has then to be removed by downward displacement. The inflection in the record from some thermocouples is probably due to the pocket of air being displaced past them in a downward direction.

It is evident from Figs. 1 and 2 that even at 20 mm. Hg abs., steam does not consistently penetrate to achieve sterilising conditions. This can be accounted for by the presence of residual air. When pumping was continued, as shown in Fig. 3, more consistent results were obtained from further removal of air, but there were still variations. It was noted that the best conditions were usually accompanied by a fall in temperature of the dressings during the latter stages of the preliminary vacuum period. This suggested that moisture was being removed as vapour and with it entrained air. Varying the moisture content and the initial temperature of the dressings produced no consistent cooling during the preliminary vacuum period and had no influence on the

temperatures obtained when steam was admitted to the chamber. The cooling effect was much increased after steam was admitted during the preliminary vacuum period to heat and moisten the fabrics. Irrespective of the load or method of packing, all parts of the pack reached sterilising temperature and at the same rate as the temperature of the chamber drain when this procedure was employed. We believe that this effect is due to moisture being distilled from the warmed and moistened fabrics under the influence of the vacuum, thereby displacing residual air so that steam when introduced for the sterilisation period penetrates rapidly into the interstices. Water vapour is the only constituent of the continuous phase. The amount of preliminary steaming, although not critical, varies with the load, and the fabrics should be raised to at least 40°. When this cycle is used the drain temperature is a true indication of the conditions within the dressings. Thus it has not been possible to obtain sterilising conditions consistently within packs of fabrics when employing the B.S. 3220/1960 cycle. We would therefore recommend the following cycle as an alternative to that set out in B.S. 3220/1960: evacuate to 20 mm. Hg abs., admit steam to 200 mm. Hg abs., evacuate to 20 mm. Hg abs., steam to 2·11 kg./cm.^{2} gauge for 3 min., evacuate to 40 mm. Hg. abs., admit air.

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

Dressings kept under normal storage conditions retained sufficient moisture to allow effective sterilisation; those kept in an arid atmosphere or with 20 per cent or more of moisture were unsuitable for the procedure. Using the technique described in the paper, holding the vacuum was considered unnecessary. The caskets used were of standard design.

IMPROVEMENT OF HEATING OF BOTTLED FLUIDS DURING AUTOCLAVE STERILISATION USING LOW PRESSURE STEAM

By G. R. Wilkinson and F. G. Peacock

From the Research Division, Allen & Hanburys Limited, Ware

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Introducing turbulent steam can improve the rate and evenness of heating large numbers of bottles in a steriliser. Experimental data is given for a charge of over 200 × 1 litre bottles in a rectangular autoclave.

To sterilise bottled fluids it is necessary to heat and maintain the fluid at 115° for 30 min. or 121° for 15 min., or for an equivalent period at other suitable temperatures (Perkins, 1956). The heating occurs as steam condenses on the bottles, and the rate of heating is influenced by the rate of transfer through the glass and by the presence of air within the autoclave. It has been thought best to remove the air by introducing the steam near the top so that it displaces the air downward and out at the lowest point of the autoclave. In a large steriliser for fluids containing many bottles this method results in grossly uneven heating of the load. We report a method of attaining uniform heating.

Experimental and Results

An unjacketed cylindrical autoclave, 122 cm. × 114 cm. diameter, loaded with 200 bottles each containing 1 litre of solution or 400 bottles each containing 500 ml. of solution, in 3 layers was used. Selected bottles were fitted with a pocket into which a thermocouple was inserted to about the centre of the liquid (Barson, Peacock, Robins and Wilkinson, 1958). Thermocouples were also positioned in the top of the chamber and the drain. Steam was introduced along the top of the autoclave and distributed to cause little turbulence. Air was eliminated through the vent which was closed when the drain temperature reached 100°.

A jacketed rectangular autoclave, 92 × 66 × 132 cm. deep, built to B.S. 1500/1960, was also used, fitted with a steam trap and a full bore vent, loaded with 260 bottles each containing 1 litre of aqueous solution, in four layers separated by perforated metal sheets. Steam was admitted from between the liner and the chamber wall through twelve holes, each 2·5 cm. diameter, in the liner near the top of the chamber, six along each side.

Tests on both autoclaves showed that the temperature variation among the bottles in any given layer was small. The temperature of one bottle at the centre of each layer was therefore chosen to represent the layer. Variation in temperature between layers was large. Results similar to those seen in Fig. 1 were obtained. Chamber temperature was reached first in the top layer then in successive lower layers. The lowest layer took some 25–30 min. longer than the top layer to attain chamber temperature.

When a loaded autoclave was evacuated to 20 mm. Hg abs. before admission of steam, the variation of temperature between layers was eliminated, as shown in a typical trace in Fig. 2. The route of steam

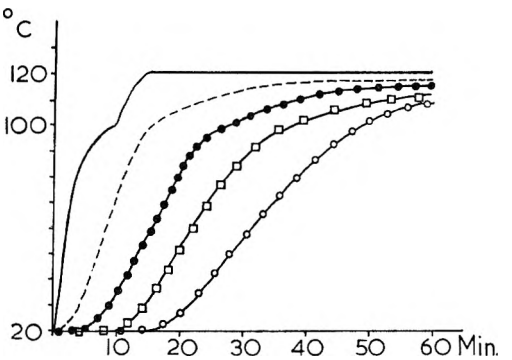

FIG. 1. Temperature distribution in bottles before turbulence of air and steam.
—— chamber. – – – 4th layer (top) of bottles. -●-●- 3rd layer of bottles.
-□-□- 2nd layer of bottles. -○-○- Bottom layer of bottles.

distribution was examined visually and a number of steam inlets constructed to produce turbulence of the steam/air mixture within the autoclaves, care being taken to ensure the aggregate area of the apertures was equal to the cross-sectional area of the supply pipe.

The effects of the following types of steam distributors were examined:

A sparge pipe with 16 holes evenly spaced along the upper and lower surfaces on the horizontal diameter near the side of the cylindrical autoclave.

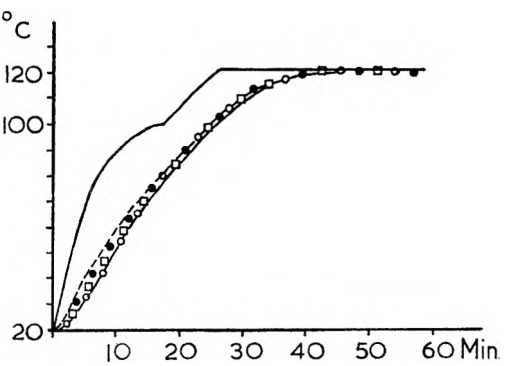

FIG. 2. Temperature distribution in bottles after improved steam distribution.
See Fig. 1 for key.

A similar pipe near the top of the vessel with its apertures directed horizontally.

Two sparge pipes along the angles between the sides and top of the rectangular autoclave, each having 60 holes each directed downwards.

A similar system with 15 holes.

A fishtail type nozzle at the centre of the back of the autoclave directed upwards.

A similar system directed downwards.

With all the modifications the bottles were heated uniformly and gave temperature traces almost identical with those shown in Fig. 2.

The behaviour of the fishtail system directed upwards was marginally superior to the others and proved the simplest in construction.

Discussion

When admitted without turbulence the steam forms a layer above the cold air, encountering at first only the upper layer of bottles, upon which it condenses. Pressure does not build up within the autoclave because of this immediate condensation of the steam. As the bottles heat up the rate of condensation upon them decreases and the layer of steam gradually increases in depth and comes into contact with the other layers of cold bottles successively, thereby heating them. The air is slowly displaced from the autoclave by the increasing volume of steam.

When the steam is introduced with turbulence a much improved distribution of heating is obtained. Steam is able to reach the surface of all bottles equally. Although air is present in the chamber, complete condensation of the steam still takes place, and the air gradually separates and is displaced through the drain during the heating up period. As before, no pressure is developed within the autoclave until all the air has been displaced. This occurs before the fluid in the bottles reaches 100°.

References

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Wilkinson, G. R., Peacock, F. G. and Robins, E. L. (1960). *J. Pharm. Pharmacol.*, **12**, *Suppl.*, 197*T*–202*T*.

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

The introduction of steam in a turbulent manner reduced the number of breakages provided steam did not impinge directly on the bottles. Steam must not be admitted faster than needed to give a condensation rate consistent with a safe rate of heat transfer across the glass of the containers. The site of the outlet is not important when turbulent steam is introduced.

A SLOPE-RATIO DESIGN FOR ACCELERATED STORAGE TESTS

By J. P. R. Tootill

From Glaxo Laboratories Limited, Greenford, Middlesex

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An experimental design is presented for predicting the "expiry" dates of pharmaceutical preparations at various storage temperatures on the basis of the degradation rates observed in short time studies at elevated temperatures. The design is based on the observation that a suitable metameter of potency falls off linearly with time at a rate depending on the absolute temperature, in accordance with the Arrhenius equation. It is particularly applicable when relatively imprecise assay methods have to be employed. The design is so constructed to involve the minimum number of time-temperature combinations necessary to check the validity of the assumptions made in applying the Arrhenius equation, and also to provide a precise estimate of known error for the expiry date.

In a series of papers Garrett, (1956a, b); Garrett and Carper, (1955) and McLeod, Pelletier and Campbell (1958) have described how, for a range of vitamins in various pharmaceutical preparations, expiry dates at storage temperatures may be quantitatively predicted on the basis of short term degradation studies at elevated temperatures. Briefly the method is based on the observation that a suitable metameter of the potency (usually the potency itself or its logarithm) falls off, at any chosen temperature, linearly with time; further, the different rates of degradation of the potency (or its metameter) obtained at different temperatures are related to these temperatures in accordance with Arrhenius' equation, that is, the logarithms of the degradation rates vary linearly with reciprocal absolute temperature. As a result of these relationships the degradation rate at storage temperature can be calculated from the experimentally determined degradation rates at elevated temperatures. These rates, being much faster, require only a short time for their determination.

However, the techniques advocated by these authors are not wholly suitable where imprecise analytical methods, such as microbiological assay, have to be used. In this context we are not concerned so much to explore the nature of the mathematical model to which the results conform, as to determine the expiry date with the highest possible precision by the assay method employed, using a model assumed to hold on the basis of pilot or published work. Observations not related to this objective should preferably be kept to the minimum required to ensure that the assumed model does hold for the preparation under study. The large number of unreplicated sampling times for each temperature (McLeod, Pelletier and Campbell (1958) recommend at least 8) is a disadvantage, as it detracts from the precision of the final estimate. Moreover it provides no objective test for the linearity of the degradation curves, since there is no independent check of the experimental error against which to test the failure of the observed points to fall exactly on a straight line. In addition, no use is made of the error of the determined degradation

constants either in fitting them to the Arrhenius equation or in calculating the error of the final estimate of expiry date. Instead, a second statistical analysis is performed on the results of the first, that is, on the logarithms of the estimated degradation constants (which in any event are generally of different precision), and their experimental error is determined by their failure to fit exactly the Arrhenius equation. Thus, unless many different temperatures are used, the final limits for the expiry date are unnecessarily widened, owing to the restricted degrees of freedom.

The purpose of this paper is therefore to show how, by a careful choice of experimental design, temperatures and times, these difficulties may be overcome. Provided only that an approximate provisional estimate,

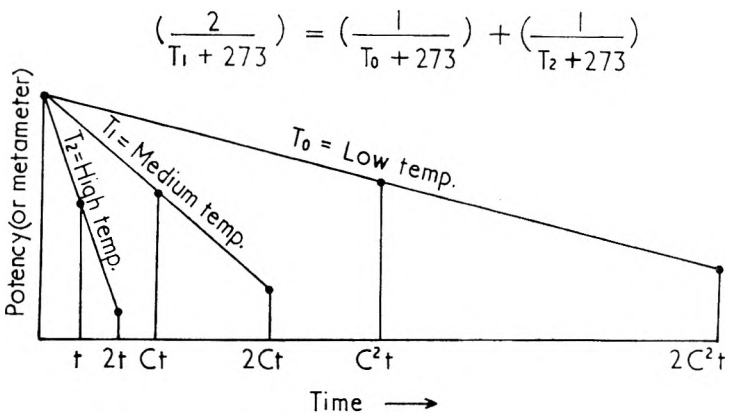


FIG. 1. The experimental design.

either from published or pilot work, of the temperature-decomposition rate relationship is available, a precise determination with known error of the storage life can be obtained from the procedure given in this paper.

Design Recommended

The design recommended involves determining the potency initially and at each of 6 time-temperature combinations, and resembles the slope ratio assay discussed by Finney (1952). It is best illustrated graphically as in Fig. 1.

The experiment is carried out at three different temperatures T_0, T_1 and T_2, so chosen that their reciprocal absolute values are in arithmetical progression. Because of this, the slopes of the three decay lines b_0, b_1 and b_2 will, if the Arrhenius relationship holds, be in geometric progression, the provisional estimate of the slope ratio (b_2/b_1, or b_1/b_0) being C. The time scales at each temperature are also in geometric progression in the opposite direction, the maximum time being 2t at T_2, 2Ct at T_1 and $2C^2t$ at T_0. Thus, provided the provisional estimate of C is reasonably accurate, approximately the same amount of degradation will have taken place by the end of the experiment whatever the temperature. Samples

are also withdrawn for assay at half these maximum times, t at T_2, Ct at T_1 and C^2t at T_0.

For a basic replication of $r > 1$, the contents of r containers of the substance under study are assayed at the beginning of the experiment. These containers should be identical to those in which the product will be normally stored. For each temperature there is a thermostatically controlled bath with 2 r containers. At time t for T_2, Ct for T_1 and C^2t for T_0, r containers are withdrawn from each bath and their contents assayed, those of the remaining r containers being assayed at time 2t for T_2, 2Ct for T_1 and $2C^2t$ for T_0.

Although the time scales at the three temperatures are not the same, at any one temperature there are two times at which the contents of containers are assayed and this allows the linearity of the decay lines to be assessed by means of the "blanks" and "intersection" terms of the normal slope ratio assay described by Finney (1952).

Finally, the adjustment of the time scales in accordance with the provisional estimate of C, the constant characteristic of the substance under study, implies that each slope (b_0, b_1 and b_2) is estimated with approximately equal precision. As will be shown later, this considerably simplifies both the subsequent statistical analysis and the test for conformity of the results with the Arrhenius model.

Factors Affecting Accuracy and Precision

It is clearly essential for the accuracy of the experiment that the temperatures be set accurately at the values chosen and that the thermostatic control mechanisms are capable of holding these temperatures precisely at these set values. The analytical method employed for assaying the withdrawn containers must also be accurate, if not precisely reproducible, and, to ensure that decomposition continues in any given container only for the time specified, these should be withdrawn at the specified time, chilled immediately and maintained at a low temperature until their contents can be assayed.

In theory, the most precise results will be obtained by setting the high temperature as high as possible and the low temperature as low as possible and by maintaining the containers at each temperature for that length of time which results in maximum decomposition at the second sampling time ($2C^2t$ at T_0, 2Ct at T_1 and 2t at T_2). In practice the high temperature is limited by the presence of volatile constituents in the product and will normally be below 100°. The choice of low temperature is limited by the maximum length of time it is proposed to devote to the experiment, for too low a temperature would require an inordinately long time for appreciable decomposition. In practice it is also preferable to aim at 50 per cent decomposition at the second sampling time as this allows leeway for errors in the provisional estimates of the decomposition rates. Failure to make such allowance might result either in a final potency so low that the analytical method employed is no longer reliable, or even in total decomposition at some unknown time before withdrawal of the containers for assay.

Choice of Convenient Values for Temperatures and Times

The considerations outlined above lead to a choice of time and temperature combinations that will yield a result as precise as possible in the light of the total time to be devoted to the experiment, the assay method employed, the safety margin for errors in the provisional estimates and the nature of the product under study as indicated by published or pilot work. The times and temperatures so indicated, however, may be far from convenient, the temperatures all being recurring decimals and the times involving withdrawal for assay in the middle of the night or at week-ends.

Because of the leeway implied by aiming at 50 per cent decomposition, a limited adjustment of the combinations indicated above is possible. But any modified choice of combinations should be checked against the provisional estimates to ensure that there is no danger of total decomposition at any temperature before the end of the experiment and that the maximum time is still not longer than that allotted to the experiment.

For temperatures, it will normally suffice to select the nearest of the four sets set out below. They all consist of three temperatures whose reciprocal absolute values are in arithmetical progression and none involves a setting finer than 0·5°.

T_0° C	39	42	52	60
T_1° C	63	63	64·5	69
T_2° C	91	87	78	78·5

The temperatures having thus been chosen, the provisional value of C can be determined from the provisional estimates. If C is expressed approximately as the improper fraction p/q where p and q are relatively prime integers, it can be seen that the times for maintaining the samples at the various temperatures will be as follows.

Temperature	1st Withdrawal	2nd Withdrawal
T_0	Kp^2 hr.	$2Kp^2$ hr.
T_1	Kpq hr.	2Kpq hr.
T_2	Kq^2 hr.	$2Kq^2$ hr.

If K is also an integer, these times will all be an integral number of hr.

However, only certain integral values for K will lead to values for these times such that the containers inserted in the constant temperature baths between 9 a.m. and 5 p.m. will be due for withdrawal also between 9 a.m. and 5 p.m. Such permissible values of K can be found as follows.

(1) From the product pq deduct the nearest multiple of 24 to yield a number (pq − 24M), positive or negative, whose value, ignoring sign, lies in the range 0–12 inclusive.

(2) If either p^2 or q^2 (or both) should differ from a multiple of 24 by unity, the permissible values for K can be read from column one of Table I against the appropriate entry $\pm$(pq − 24M).

(3) If neither p^2 nor q^2 should differ from a multiple of 24 by unity, the permissible values of K are read from column two of Table I.

(4) For any permissible value of K, the values 24N $\pm$ K, where N is an integer, are also permissible.

These calculations ensure that each sampling time occurs within $\pm$8 hr. of an integral number of days after the insertion of the containers in the thermostatically controlled bath. Since 9 a.m. to 5 p.m. on the same day

TABLE I

$\pm$(pq − 24M)	Column one	Column two
0	1, 2, 3, 4, 8	3, 5, 8, 11
1	1, 2, 3, 4, 8	
2	1, 2, 4, 8	
3	8	
4	1, 2, 4, 8	
5	4, 8	
6	4, 8	8
7	3, 4, 8	
8	1, 2, 3, 4, 8	
9	3, 8	
10	2, 4, 8	
11	2, 4, 8	
12	2, 4, 8	8

is +8 hr. and 5 p.m. to 9 a.m. on the following day is 1 day − 8 hr., it is evident that the only remaining problem is to ensure that the integral number of days involved in each interval does not result in week-end work. This can always be achieved by expressing the number of days as w weeks + d days where d will be in the range $\pm$4 days. Table II gives the value of d for various combinations of insertion and withdrawal days. Thus, for example, if the first sampling time at a given temperature were

TABLE II

		Withdrawal from bath w weeks later, on				
		M	Tu	W	Th	F
Insert in bath, on:	M	0	+1	+2	+3	−4
	Tu	−1	0	+1	+2	−3
	W	−2	−1	0	+1	+2
	Th	−3	−2	−1	0	+1
	F	−4	−3	−2	−1	0

280 hr. this would be expressed as 2 weeks − 2 days − 8 hr. The second sampling time is then 560 hr. which would be expressed as 3 weeks + 2 days + 8 hr. Thus the set of containers for the 280 hr. sampling time might be inserted in the bath at 5 p.m. on a Thursday and withdrawn at 9 a.m. on the Tuesday 12 days later. Those for the 560 hr. sampling time could be inserted at 9 a.m. on Monday and withdrawn at 5 p.m. on the Wednesday 23 days later.

Application

Choice of experimental conditions. A certain vitamin B_{12} preparation with an initial potency of about 1,000 u/ml. was used. The potency was determined microbiologically with *Escherichia coli* M200 by the method of Harrison, Lees and Wood (1951), and in preliminary trials this was found graphically to fall about 0·217 u/ml./hr. at 51·5° and 1·96 u/ml./hr.

at 70°. It was proposed that the maximum time to be devoted to the examination of the product at elevated temperatures should be about 4 weeks (720 hr.). Graphical interpolation on a logarithmic plot of the above provisional degradation rates against reciprocal absolute temperatures showed that a rate of 0·694 u/ml./hr. (i.e. to give half the potency 1,000 u/ml. in 720 hr.) was likely to be achieved at a temperature of 61°. It was, therefore, decided to investigate the possibilities of using the final set of temperatures tabulated in the preceding section. The graph gave the provisional degradation rates:

Temperature ° C	Provisional Rate
60	0·615 u/ml./hr.
69	1·751 u/ml./hr.
78·5	4·985 u/ml./hr.

From this, the provisional value of C = 2·8. As an approximation this was taken to be 8/3 = 2·66667, i.e. p = 8, q = 3.

TABLE III

Containers put in bath	Containers withdrawn	Time
Wed. 21st Sept. 9 a.m.	Tues. 4th Oct. 5 p.m.	320 hr.
Wed. 21st Sept. 5 p.m.	Tues. 18th Oct. 9 a.m.	640 hr.
Wed. 28th Sept. 5 p.m.	Mon. 3rd Oct. 5 p.m.	120 hr.
Tues. 27th Sept. 3 p.m.	Fri. 7th Oct. 3 p.m.	240 hr.
Tues. 18th Oct. 5 p.m.	Thurs. 20th Oct. 2 p.m.	45 hr.
Fri. 7th Oct. 4 p.m.	Tues. 11th Oct. 10 a.m.	90 hr.

Since neither 8^2 nor 3^2 differs from a multiple of 24 by unity, the value $(3 \times 8 - 24) = 0$ was read off column two in Table I and the values K = 3, 5, 8, 11 found permissible. Taking K = 5, the times at the various temperatures were calculated as follows.

Temperature ° C	1st Withdrawal	2nd Withdrawal
60	Kp^3 = 320 hr.	$2Kp^2$ = 640 hr.
69	Kpq = 120 hr.	2Kpq = 240 hr.
78·5	Kq^2 = 45 hr.	$2Kq^2$ = 90 hr.

These times were achieved according to the schedule in Table III.

Results. At the end of the experiment the triplicate results given in Table IV were obtained (r = 3). The various figures Z = 2,821, etc., are used in subsequent calculations explained below.

Calculation of treatment effects and error. The first stage in the calculation involves summing the replicate results for each time-temperature combination. Thus:

$$Z = 987 + 864 + 970 = 2{,}821,$$
$$L_0 = 724 + 702 + 769 = 2{,}195,$$
$$M_0 = 447 + 585 + 638 = 1{,}670, \text{ etc.}$$

These totals are entered in the appropriate column and are then summed to yield the grand total:

$$G = Z + L_0 + M_0 + L_1 + M_1 + L_2 + M_2 = 13{,}952.$$

The total variation among results is given by the sum of the squares of all individual results from which is subtracted $G^2/7r$. Thus:

$987^2 + 864^2 \ldots + 506^2 - 13{,}952^2/21 = 452{,}575$ for $(7r - 1) = 20$ degrees of freedom.

The variation due to treatments (i.e. time-temperature combinations) is given by:

$(Z^2 + L_0^2 + M_0^2 + L_1^2 + M_1^2 + L_2^2 + M_2^2)/r - G^2/7r = 412{,}641$ for 6 degrees of freedom.

TABLE IV

Time hr.	Temp. °C	Replicate results				
0	—	987 864 970	$Z = 2{,}821$			
320 640	60 60	724 702 769 447 585 638	$L_0 = 2{,}195$ $M_0 = 1{,}670$	$P_0 = 2{,}720$ $Q_0 = 5{,}535$	$1 = 1{\cdot}000{,}000$	
120 240	69 69	668 735 701 549 462 522	$L_1 = 2{,}104$ $M_1 = 1{,}533$	$P_1 = 2{,}675$ $Q_1 = 5{,}170$	$x = 1{\cdot}058{,}000$	$1 = 1{\cdot}000$
45 90	78·5 78·5	724 683 652 531 533 506	$L_2 = 2{,}059$ $M_2 = 1{,}570$	$P_2 = 2{,}548$ $Q_2 = 5{,}199$	$x^2 = 1{\cdot}119{,}364$	$2x = 2{\cdot}116$
			$G = 13{,}952$ $H = 66{,}144$	$\Sigma P = 7{,}943$ $\Sigma Q = 15{,}904$	$3(1 + x + x^2) = 9{\cdot}532{,}092$	$3(1 + 2x) = 9{\cdot}348$

The variation due to error is obtained by difference

$452{,}575 - 412{,}641 = 39{,}934$ for $7(r - 1) = 14$ degrees of freedom.

The analysis so far is summarised:

Source of variation	D.F.	Squares	Variance
Total variation	20	452,575	
Treatments	6	412,641	
Error	14	39,934	2,852

The variance, obtained by dividing the squares by the corresponding number of degrees of freedom, need only be evaluated for the error term.

Subdivision of treatment effects. The treatment effects calculated above are then further subdivided into terms relevant to the linearity of the decay curves and to the rates of decay observed.

The former terms are derivable from the P values at each temperature where $P = 2L - M$. The "intersection" term for 2 degrees of freedom is given by $\Sigma P^2/5r - (\Sigma P)^2/15r = 1{,}061$ and represents the variation due to the failure of the three decay curves, when continued back to zero time, to meet at a common point. The "blanks" term is obtained from $(\Sigma P - 3Z)^2/24r = 3{,}756$ for 1 degree of freedom and represents the variation due to the failure of the average of the three intersection points to coincide with the average initial potency.

The terms last mentioned are derivable from the Q values at each temperature where $Q = L + 2M$. The values of the slopes of the decay lines, each expressed on its own time scale, i.e. per $C^2t = 320$ hr. at $T_0 = 60°$ C, per $Ct = 120$ hr. at $T_1 = 69°$ C and per $t = 45$ hr. at

$T_2 = 78{\cdot}5°$ C, will not be expected to be the same unless the provisional value of C happens to correspond with the true value. The variation due to differences between these slopes is given by $\Sigma Q^2/5r - (\Sigma Q)^2/15r = 5{,}488$. The variation due to the average decay recorded during the experiment is given by $(9G - 7\Sigma Q)^2/168r = 402{,}336$.

This stage of the analysis is summarised:

Source of variation	D.F.	Squares	Variance
Intersections	2	1,061	530
Blanks	1	3,756	3,756
Slope differences	2	5,488	
Mean slope	1	402,336	
Treatments	6	412,641	

It will be observed that these terms sum to the squares previously calculated for the variation due to treatments. The variance terms for intersections and blanks should be obtained by dividing the corresponding squares by 2 and 1 degrees of freedom, respectively. If neither is significant when compared with the error variance, it may be assumed that, within experimental error, the three decay curves are linear, as required by the model on which the experiment is based.

Attribution of the slopes to the Arrhenius equation. The values of the slopes at each temperature b_0, b_1 and b_2, each in terms of its own time scale, may be obtained as shown below.

$B_0 = 40rb_0 = H - 8Q_0 = 21{,}864$ therefore $b_0 = 182{\cdot}2$ u/ml./320 hr.
$B_1 = 40rb_1 = H - 8Q_1 = 24{,}784$ therefore $b_1 = 206{\cdot}5$ u/ml./120 hr.
$B_2 = 40rb_2 = H - 8Q_2 = 24{,}552$ therefore $b_2 = 204{\cdot}6$ u/ml./ 45 hr.
where $H = 15G - 9\Sigma Q = 66{,}144$.

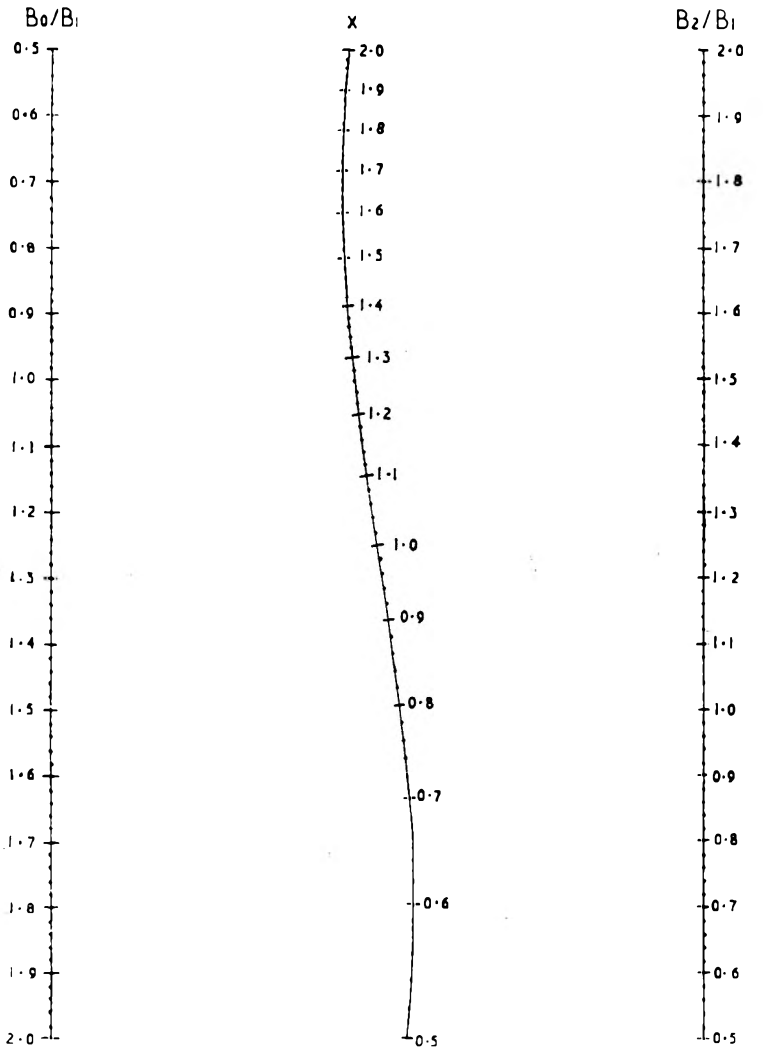
If the true value of the slope ratio is Cx, whereas the provisional value chosen for the experiment is C, then the ratio of b_1/b_0 and b_2/b_1 should be x. In practice, even if the Arrhenius model holds good, these two slope ratios will not be exactly the same owing to experimental error. The least squares value for x can, however, be obtained to an accuracy of about 0·002 from the nomogram. The values $B_0/B_1 = 0{\cdot}882$ and $B_2/B_1 = 0{\cdot}991$ are calculated and the nomogram is read, yielding $x = 1{\cdot}058$.

From this value of x is column containing 1 against Q_0, x against Q_1 and x^2 against Q_2 is entered, three times the sum of this column, i.e. $3(1 + x + x^2)$ being entered at the foot of the column. Similarly, a second column containing 1 against Q_1 and 2x against Q_2, together with three times its sum, i.e. $3(1 + 2x)$ at the foot, is also constructed. The corresponding total used for the Q column is not ΣQ but G the grand total of all results:

Q_0 Q_1 Q_2	1 x x^2	1 2x
G	$3(1 + x + x^2)$	$3(1 + 2x)$

By utilising only the entries involving x the following F functions may readily be calculated.

$$B_0(9x^4 - 43x^3 + 27x^2 - 17x) - B_1(17x^4 - 18x^3 + 18x - 17) + B_2(17x^3 - 27x^2 + 43x - 9) = 0$$



NOMOGRAM

From the row opposite Q_2:

$$D = 175[1 + (2x)^2 + (x^2)^2] - 10[3(1 + x + x^2)]^2$$
$$= 175[1 + 2{\cdot}116^2 - 1{\cdot}119,364^2] - 9{\cdot}532,092 \times 95{\cdot}320,920 = 269{\cdot}217,78$$

From the final column:

$$F_1 = \frac{35[1 + (2x)^2] - [3(1 + 2x)]^2}{D}$$

$$= \frac{35[1 + 2{\cdot}116^2] - 9{\cdot}348^2}{269{\cdot}217,\ 78} = 0{\cdot}387{,}514,\ 7$$

From the corresponding entries of the final two columns:

$$F_2 = -\frac{35[x + (x^2 \times 2x)] - [3(1 + 2x) \times 3(1 + x + x^2)]}{D}$$

$$= -\frac{35[1{\cdot}058 + 2{\cdot}116 \times 1{\cdot}119{,}364] - 9{\cdot}348 \times 9{\cdot}532{,}092}{269{\cdot}217,\ 78}$$

$$= -0{\cdot}114{,}495,\ 0$$

From the penultimate column:

$$F_3 = \frac{35[1 + x^2 + (x^2)^2] - [3(1 + x + x^2)]^2}{D}$$

$$= \frac{35[1 + 1{\cdot}058^2 + 1{\cdot}119{,}364^2] - 9{\cdot}532{,}092^2}{269{\cdot}217,\ 78}$$

$$= 0{\cdot}100{,}926,\ 1$$

By operating on the Q column the following R functions may be obtained:

$$R_1 = \frac{1}{7}G \times 3(1 + x + x^2) - (Q_0 + xQ_1 + x^2Q_2)$$

$$= \frac{13{,}952 \times 9{\cdot}532{,}092}{7} - (5535 + 5170 \times 1{\cdot}058 + 5199 \times 1{\cdot}119{,}364)$$

$$= 2174{\cdot}388$$

$$R_2 = \frac{1}{7}G \times 3(1 + 2x) - (Q_1 + 2xQ_2)$$

$$= \frac{13{,}952 \times 9{\cdot}348}{7} - (5{,}170 + 5199 \times 2{\cdot}116)$$

$$= 2{,}460{\cdot}815$$

With the aid of these functions we may now calculate the least squares value, β_0, of the slope at $T_0 = 60°$, assuming the Arrhenius model to hold, and the correction, $r\beta_0\Delta x$, to be applied to the nomographic value of x (which, as mentioned above, may be in error by about 0·002).

$$r\beta_0 = F_1R_1 + F_2R_2 = 0{\cdot}387{,}514,\ 7 \times 2{,}174{\cdot}388 - 0{\cdot}114{,}495,\ 0 \times 2{,}460{\cdot}815$$

$$= 560{\cdot}856 \text{ whence } \beta_0 = 186{\cdot}95 \text{ u/ml./320 hr.}$$

$$r\beta_0\Delta x = F_2R_1 + F_3R_2 = -0{\cdot}114{,}495,\ 0 \times 2{,}174{\cdot}388 + 0{\cdot}100{,}926,\ 1 \times 2{,}460{\cdot}815$$

$$= -0{\cdot}596 \text{ whence } \Delta x = -\frac{0{\cdot}596}{560{\cdot}856} = -0{\cdot}00106$$

$$\text{whence } x = 1{\cdot}056,\ 94$$

The variation attributable to the Arrhenius model is given by

$$\frac{r\beta_0 \times R_1 + r\beta_0\Delta x \times R_2}{r} = \frac{560{\cdot}856 \times 2{,}174{\cdot}388 - 0{\cdot}596 \times 2{,}460{\cdot}815}{3}$$

$$= 406{,}017 \text{ for 2 degrees of freedom.}$$

The total variation due to the 3 degrees of freedom for slopes, is obtained by adding that due to the 2 degrees of freedom for slope differences 5,488 to that for the single degree of freedom for mean slope 402,336, calculated under 'sub-division of treatment effects': this gives 407,824. The difference 407,824 − 406,017 = 1,807 for 1 degree of freedom represents the departure of the results from the Arrhenius model.

These findings are summarised:

Source of variation	D.F.	Squares	Variance
Slopes	3	407,824	
Arrhenius model	2	406,017	
Deviation	1	1,807	1,807

Only the variance for deviation from the Arrhenius model need be considered; if this is not significant by comparison with the error variance, it may be taken to justify the assumption that the slopes are in geometric progression within experimental error.

Calculation of degradation rate at any specified temperature. For any specified temperature T the value y may be calculated as follows:

$$y = -\frac{(273 + T_1)(T_0 - T)}{(273 + T)(T_1 - T_0)}$$

Thus if T = 25° for $T_0 = 60°$, $T_1 = 69°$

$$y = -\frac{(273 + 69)(60 - 25)}{(273 + 25)(69 - 60)} = -4{\cdot}4631$$

The slope β_y for this value of T may be calculated from

$$\log \beta_y = \log \beta_0 - 4{\cdot}4631 \log Cx.$$

With $\beta_0 = 186{\cdot}95$ u/ml./320 hr., C = 2·6667 and x = 1·056, 94, this yields $\beta_y = 1{\cdot}8334$ u/ml./320 hr. at 25°.

The standard error of this estimate is given by

$$\text{s.e. } (\beta_y) = \sqrt{\frac{\text{error variance}}{r}} \times \frac{\beta_y}{\beta_0} \times \sqrt{F_1 + 2\left(\frac{y}{x}\right) F_2 + \left(\frac{y}{x}\right)^2 F_3}$$

which as y/x = −4·222, 65 is in the above case,

$$\text{s.e. } (\beta_y) = \pm\sqrt{\frac{2{,}852}{3}} \times \frac{1{\cdot}8334}{186{\cdot}952} \times$$

$$\sqrt{0{\cdot}387,514,7 + 2 \times 0{\cdot}114,495,0 \times 4{\cdot}222,65 + 0{\cdot}100,926,1 \times 4{\cdot}222,65^2}$$

$$= \pm 30{\cdot}833 \times 0{\cdot}009,806,8 \times 1{\cdot}775,96$$

$$= \pm 0{\cdot}5370$$

In practice we are concerned that the product should not decay faster than we have estimated, so that only the upper fiducial limit is of interest. This being so, the Students *t* value to be taken from the tables is that related to twice the probability value we wish to use. Thus for the 95 per cent upper fiducial limit we do not take the 5 per cent value but

rather the 10 per cent value of t for $7(r - 1)$ degrees of freedom. In the above example, for 14 degrees of freedom $t = 1{\cdot}761$, hence the 95 per cent upper fiducial limit for the decay at 25° is $\beta_y + 1{\cdot}761$ s.e. $(\beta_y) = 1{\cdot}8334 + 1{\cdot}761 \times 0{\cdot}5370 = 2{\cdot}7791$ u/ml./320 hr. Hence if we require that the product lose not more than 20 per cent of its nominal initial value of 1,000 u/ml., i.e. not more than 200 u/ml., the maximum life for $P = 0{\cdot}95$ at 25° $= 200/2{\cdot}7791 \times 320$ hr. $= 23{,}029$ hr. or about 32 months.

Acknowledgement. The author wishes gratefully to acknowledge the experimental work of Mr. J. Bell which has enabled the feasibility and precision of the design to be tested in practice.

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

The method was designed for products that obeyed the Arrhenius equation, and included validity tests which showed up non-conforming preparations at an early stage of the calculations. Estimations of the degree of error permissible in the times of measurement and the temperature of the test were dependent upon the products to be tested. It was necessary to avoid very short times, and a statistician could make allowance for deviations from the required temperatures if such deviations were known.

ETHYLENE OXIDE STERILISATION—SOME EXPERIENCES AND SOME PRACTICAL LIMITATIONS

By A. Royce and C. Bowler

From the Microbiology Division, Standards Department, Boots Pure Drug Co. Ltd., Nottingham

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Causes of failure to sterilise using ethylene oxide have been investigated. Organisms can occur naturally in conditions in which they are "protected" from the action of the gas, and in consequence its action on organisms which have been artificially protected has been studied. The work is discussed with particular reference to moisture content and relative humidity, and means of overcoming the difficulties of sterilising "protected" organisms are suggested. Bacteriological controls have been found unreliable without safeguards.

Since the work of Phillips and Kaye (1949) many papers have been published on ethylene oxide sterilisation. Most have attested its effectiveness, but Walter and Kundsin (1959), Barwell and Freeman (1960) and Znarmirowski, McDonald and Roy (1960) reported that sterilisation of test objects was not always obtained. Similar cases of non-sterilisation of various materials and bacteriological controls occurring over the last 10 years are summarised in this paper and we have given some account of the laboratory investigations connected with them.

Many inter-related factors govern an ethylene oxide sterilisation. These include the time concentration product of the gas, the temperature and the relative humidity, the moisture content of the materials, the sorptive capacity of the materials, and the accessibility of the organisms to the gas.

Almost every worker has his own combination of the inter-related variables, time, gas concentration and temperature. Some treatments advocated in the literature are many times more stringent than others. Royce and Bowler (1959) suggested sorption of the gas can account for some of the variations in treatment practiced.

Kaye and Phillips (1949) noted that organisms dried from salt solutions were more difficult to sterilise than those dried from water, and that organisms dried on hard impervious surfaces were more difficult than those dried on absorbent surfaces. This they attributed to dry crust formation composed of protein or salts which prevented access of the gas to the organisms. Royce and Sykes (1955) mentioned that organisms in dried broth films were not always sterilised and again Royce (1959) drew attention to the fact that micro-organisms could be trapped inside crystals of various substances and were thus protected from ethylene oxide sterilisation. Abbott, Cockton and Jones (1956) working with bacterial spores noted that their inclusion in the crystals of many substances protected them from sterilisation by formaldehyde and ethylene oxide.

Experimental

Ethylene oxide at 10 per cent v/v gas concentration in air at ambient temperature (approximately 20°) and humidity (45–80 per cent) and

atmospheric pressure for an exposure period of about 20 hr. was employed for most of this work. This is referred to subsequently as the "standard treatment".

Naturally occurring "protected" organisms. Initial experiments using soil-dust-spore preparations, showed efficient sterilisation by the standard treatment. Further preliminary experiments with a range of pharmaceutical powders demonstrated sterilisation in most instances, but a few batches of glucose, lactose and sulphanilamide were notable exceptions. Later some batches of soil-dust-spores also gave occasional surviving organisms. Tests showed that this was not because of loss of ethylene oxide owing to reaction with or absorption by the materials. Progressive increments in the severity of the treatment, involving raising the gas

TABLE I

THE EFFECT OF ETHYLENE OXIDE ON MICRO-ORGANISMS IN THIN DRIED FILMS OF NUTRIENT BROTH

Organisms	Viable count (orgs/piece)	
	Before treatment	After treatment
Glass rods		
Staph. aureus	2×10^4	6×10^3
B. subtilis	10^4	Nil
Cl. sporogenes	5×10^7	Nil
Glass coverslips		
Staph. aureus	2×10^4	10^3
B. subtilis	10^4	2×10^3
Cl. sporogenes	5×10^3	Nil
Filter paper strips		
Staph. aureus	+(2)	−(4)
B. subtilis	+(2)	−(4)
Cl. sporogenes	+(2)	−(4)

+ = Growth − = No growth ( ) = Number of pieces tested

concentration (up to 100 per cent and even under increased pressure), extending the exposure period (up to 3 days) and raising the temperature (up to 80°) were ineffective, except once or twice with lactose and soil-dust-spores.

Many of the surviving organisms were tested to determine their inherent resistance to ethylene oxide by drying their cultures on filter paper strips and then subjecting them to the standard treatment. The initial viable counts were in the range 10^7–10^8 cells per strip, and in every instance they were sterilised.

Artificially protected organisms on different surfaces. Experiments using artificially protected organisms revealed that those in dried nutrient broth films on glass were rarely sterilised, but the same organisms dried from serum suspension were much more susceptible. In further studies nutrient broth cultures of *Staphylococcus aureus*, *Bacillus subtilis* and *Clostridium sporogenes* were distributed (*a*) in thin films, by dipping short lengths of glass rod and carefully draining them; (*b*) in thick films, by spreading large drops on glass coverslips, and (*c*) in absorbed films on filter paper strips. The preparations were dried over phosphorus pentoxide for 24 hr. and then given a standard treatment, after which the the glass test pieces were washed in tubes of broth and viable counts

made on the broth suspensions; the strips were cultured intact. The results of these tests are given in Table I.

In similar tests cultures of organisms which had survived ethylene oxide treatment were washed off agar slopes with saline. Part of each saline suspension was filtered through a Ford Sterimat (F.C. grade), which was then dried, and the remainder was absorbed in kieselguhr, which was also dried. Six of the 9 pad preparations, but none of the kieselguhr preparations, were sterilised by the standard treatment.

A final combined experiment was made to compare broth and normal saline suspensions of the same micro-organisms deposited or dried on different surfaces. The two vegetative organisms *Staph. aureus* and *Escherichia coli* were used, 18 to 24 hr. cultures from agar slopes being

TABLE II

THE EFFECT OF ETHYLENE OXIDE ON DIFFERENT BACTERIOLOGICAL CONTROLS PREPARED FROM THE SAME CULTURES

Surface used	Number sterile/Number tested			
	Staph. aureus in		*E. coli* in	
	Broth	Saline	Broth	Saline
Glass coverslip	0/5	0/5	2/5	0/5
Asbestos Sterimat	5/6	4/6	6/6	4/6
Kieselguhr powder	4/6	5/6	5/6	5/6
Filter paper	6/6	6/6	6/6	6/6

suspended in nutrient broth or in saline and 0·2 ml. amounts separately dried on: (*a*) $\frac{3}{4}$ in. diameter glass coverslips; (*b*) filter paper strips (Whatman No. 1); (*c*) $\frac{3}{4}$ in. squares of Sterimat pads (Ford F.C. grade), and (*d*) 2 g. quantities of kieselguhr. Six replicates of each preparation were given a standard ethylene oxide treatment and subsequently cultured individually, with the results shown in Table II.

Artificially protected organisms in powders. Glucose monohydrate was infected with an aqueous suspension of *B. subtilis* spores and air dried. It was further dried to remove most of the water of crystallisation and yield a preparation consisting mainly of anhydrous glucose. Tests showed this treatment had little effect on the viable count of contaminating spores. Some of this anhydrous material was then partly rehydrated with 5 per cent water, and some was more than fully rehydrated by adding 12 per cent water. Samples of the original contaminated monohydrate, the anhydrous, the partially and fully rehydrated materials were subjected to a standard ethylene oxide treatment. Results are presented in Table III. A similar experiment using sodium citrate, which is also a hydrated crystal, gave similar results.

Procaine penicillin also yields occasional contaminants. As before, cultures of contaminants dried on filter paper strips were sterilised by the standard ethylene oxide treatment. Portions of the cultures were added to separate aqueous solutions of sodium penicillin and procaine hydrochloride, after which the contaminated solutions were mixed and the precipitated procaine penicillin was collected, washed and dried. After

7 days storage at room temperature the preparation contained 4×10^7 organisms per gram, and after standard ethylene oxide treatment yielded an average survivor count of 3×10^5 organisms per gram.

In other work saline suspensions of contaminants were ground with sodium chloride, glucose, soil and sand and air dried. This procedure produced powders having counts of 10^6–10^7 organisms per gram. After ethylene oxide treatment the soil and sand preparations yielded only 10–10^2 organisms per gram, but the glucose and sodium chloride yielded 10^4–10^5 organisms per gram.

Effect of moisture and humidity. Using the same preparations and varying the moisture content from "high dried" up to 5 to 6 per cent moisture revealed no effect with the sodium chloride and glucose, but

TABLE III

THE EFFECT OF ETHYLENE OXIDE ON MICRO-ORGANISMS IN CRYSTALLINE AND ANHYDROUS GLUCOSE

Substances	Original count (orgs./g.)	Count after treatment (orgs./g.)
Glucose monohydrate	2×10^7	$1{\cdot}5 \times 10^4$
Glucose anhydrous	3×10^6	$1{\cdot}5 \times 10^3$
Glucose anhydrous (partially rehydrated)	3×10^6	$1{\cdot}2 \times 10^4$
Glucose anhydrous (fully rehydrated)	3×10^6	$5{\cdot}5 \times 10^4$

gave a higher probability of kill with the sand and soil preparations at the higher moisture levels. Again more severe ethylene oxide treatments produced no significant improvement.

Experiments were made with gross moisture additions of 25 and 33 per cent, which converted the preparations into sludges, and then adding liquid ethylene oxide (1 per cent) after the method of Wilson and Bruno (1950) for sterilising culture media. The sand and soil preparations, with their small amounts of easily soluble crystalloids, were sterilised, but the glucose and sodium chloride sludges, which consisted largely of undissolved crystals containing organisms were not sterilised and yielded high counts after treatment: a similar experiment with procaine penicillin also yielded a high count.

In confirmation of these observations, naturally infected samples of soil-dust-spore mixture, fuller's earth and bentonite, none of which had been sterilised initially by ethylene oxide, were readily sterilised after being well washed and dried. Tests showed that very few organisms were lost by washing and viable counts both before and after washing were 10^6–10^7 organisms per gram.

Further light was thrown on the problem when attempts were made to apply the washing technique to various sulphonamides and penicillin salts of differing solubilities in water. Spore infected preparations were made by washing bacterial spores from a slope with distilled water and incorporating in the powder by trituration in a mortar and subsequent drying. Samples (except sodium penicillin) were separately washed in water, recollected and dried. Washed and unwashed materials were given standard ethylene oxide treatments. Results are in Table IV.

Contaminations which may occur on surgical instruments, glassware and apparatus are likely to be present as dried films. Some of the crystalloids present in such films may be hygroscopic, and at high humidities may pick up enough moisture to solubilise the film partially or completely. Experiments were therefore devised using moist and dry films at various humidities.

TABLE IV

THE EFFECT OF ETHYLENE OXIDE ON UNWASHED AND WASHED CONTAMINATED SULPHONAMIDES AND PENICILLIN SALTS

Substance and treatment	Initial count (orgs./g.)	Count after treatment (orgs./g.)
Sodium Penicillin (very soluble)		
Unwashed	$2{\cdot}5 \times 10^8$	$1{\cdot}5 \times 10^4$
Sulphanilamide (soluble 1/170)		
Unwashed	6×10^7	5×10^8
Washed	1×10^7	$1{\cdot}3 \times 10^8$
Procaine penicillin (soluble 1/200)		
Unwashed	8×10^8	1×10^2
Washed	$1{\cdot}8 \times 10^8$	$1{\cdot}5 \times 10^3$
Sulphathiazole (soluble 1/2,500)		
Unwashed	$1{\cdot}4 \times 10^8$	6×10^4
Washed	6×10^7	4×10^5
Benzathine Penicillin (soluble 1/6,000)		
Unwashed	2×10^8	Nil
Washed	3×10^7	Nil

TABLE V

THE EFFECT OF ETHYLENE OXIDE ON WET AND DRIED BACTERIAL FILMS ON GLASS

Preparation	Viable count (orgs./coverslip)	
	Before treatment	After treatment
B. subtilis broth culture		
Wet	$5{\cdot}4 \times 10^8$	Nil
Dried	Not done	2×10^8
B. subtilis spores in saline		
Wet	$4{\cdot}5 \times 10^7$	Nil
Dried	Not done	5×10^6
Staph. aureus broth culture		
Wet	1×10^8	Nil
Dried	Not done	2×10^6

0·1 ml. quantities of 18–24 hr. nutrient broth cultures of *Staph. aureus* and *B. subtilis* and 0·1 ml. quantities of a suspension of *B. subtilis* spores in normal saline were spread on glass coverslips. Counts were made of the various preparations. Some were dried over phosphorus pentoxide, and then treated with ethylene oxide, others were transferred immediately to the ethylene oxide chamber whilst still wet and were given an immediate treatment. All treatments were at 50–65 per cent relative humidity. The wet preparations dried out in the ethylene oxide chamber within 1 hr. Results are given in Table V.

Dried film preparations of the broth cultures of *Staph. aureus* and *B. subtilis* were variously treated before standard ethylene oxide treatment. In each experiment four *Staph. aureus* and four *B. subtilis* coverslips were treated as follows.

(*a*) Dry prepared coverslips were placed in the gas chamber, sufficient moisture was added to give slightly super-saturated conditions at 20° and

the chamber and contents were heated to 45° to vaporise the added moisture. The coverslip preparations remained hard and dry. The chamber was then cooled to ambient temperature, "dew" was precipitated in the chamber and the preparations became moist and sticky.

(*b*) Sufficient moisture was added to the chamber to produce 100 per cent humidity at 20° and the chamber and contents were allowed to stand at room temperature for 3 days to equilibrate and were not heated.

(*c*) As (*b*) but ethylene oxide was added immediately with no equilibration period. Eight *B. subtilis* spore preparations in normal saline dried on coverslips were also included in this experiment.

All the test coverslips were sterilised.

A final experiment was made using process (*a*) again but this time after "dew" had moistened the preparations, the chamber and contents were raised to 55° and given an ethylene oxide treatment at 1,500 mg./l. (approximately 75 per cent ethylene oxide v/v) for 2 hr. at 55°. (This treatment is more stringent than the standard treatment.) Relative humidity during the sterilisation period was 60–70 per cent. None of the coverslips was sterilised.

Discussion

The differences shown by the results are clearly not due to differences between organisms in their resistance to ethylene oxide; they do show that organisms can be so prepared that they are protected from the action of the gas.

It is noteworthy that serum with its high protein content fails to protect, whereas nutrient broth, with its higher content of soluble amino-acids and other crystalloids and low protein content, is a good protecting material. A compact spherical organism, *Staph. aureus*, is more easily protected than the rod shaped *E. coli* and is generally more difficult to sterilise than the larger bacterial spores. The same cultures are protected when dried on glass but not when dried on filter paper (Tables I and II).

Organisms in saline solutions can be dried on filter paper and sterilised by ethylene oxide, when dried on asbestos filter pads or kieselguhr, they are sometimes sterilised, or when dried mixed with soluble powders such as glucose or sodium chloride, they are not sterilised. Thus, whilst organisms dried on hard surfaces are more difficult to sterilise than those on absorbent surfaces, not all organisms dried on absorbent surfaces can be sterilised (Table II).

The experiments with the hydrated glucose and sodium chloride crystals indicate that the organisms are included in the crystals, and, with hydrated crystals, can be released by drying off the water of the crystallisation under mild conditions. The inclusion of organisms in other materials has been demonstrated more elegantly by Abbott, Cockton and Jones (1956) using the electron microscope. The practical significance of crystal protection is well illustrated by the occurrence of such protected organisms in materials. We have shown that vegetative organisms as well as bacterial spores can be protected in crystal preparations and can survive for some weeks under such conditions.

Bacteriological controls do not provide satisfactory proof of effective sterilisation, since, as we have indicated (Table II), they can be prepared to show either sterilisation or non-sterilisation at will.

In pharmaceutical practice, for powder sterilisation the importance of ensuring that any crystallisation or precipitation stage is carried out aseptically is clearly demonstrated with procaine penicillin. Further, washing slightly soluble substances can convert a surface contamination, which could be eliminated by ethylene oxide, into an internal one that cannot.

Thus in routine operations with ethylene oxide it is possible to sterilise successively many batches of a given material and then to encounter a a batch which contains "protected" organisms which cannot be sterilised. Increasing the stringency of the ethylene oxide treatment is usually ineffective. This implies that constant surveillance of procedures with tests on the materials treated is an essential part of ethylene oxide sterilisation.

It is however, possible to devise a means of circumventing at least some of the difficulties. Thus for insoluble powders, merely washing with water can liberate the "protected" organisms and render the material, after redrying, sterilisable by ethylene oxide.

The experiments at 100 per cent relative humidity show that organisms in dried films can be effectively sterilised. Any substantial amounts of free water should be avoided because of the high sorptive capacity of water for ethylene oxide. More work is required on this aspect.

For absorbent materials such as paper and fabrics, it appears that where a fluid containing organisms is completely absorbed by the fibres the organisms which presumably remain on the fibre surface do not become protected. If more fluid is present than can be absorbed and this dries off in the free spaces between fibres, then "protected" organisms can occur and the material cannot be sterilised by ethylene oxide. Such organisms would probably be liberated at high humidities and there is an obvious application which might be further pursued, to dressing sterilisation.

Phillips (1961) in work on moisture in ethylene oxide sterilisations has shown that for a "naked" organism the moisture content of the organism corresponding to equilibration at 33 per cent R.H. approximately is optimum for sterilisation. High moisture impedes the sterilisation process, but lower moisture very markedly interferes and induces resistance to ethylene oxide sterilisation. He has also shown that high dried organisms require extended periods of equilibration at high humidities to make them susceptible again to ethylene oxide or, alternatively, require to be physically wetted, when they are speedily rendered sterilisable by ethylene oxide.

We agree with Phillips conclusions, but in practice protected organisms can occur. If organisms are enclosed inside crystals of insoluble materials, no equilibration at any relative humidity will affect them and they remain unsterilisable. With water soluble crystals, freeing of the organisms can take place if the partial pressure of water vapour exceeds the vapour pressure of a saturated solution of the substance enclosing the organism. In such cases, or if they are physically wetted as, for example, by "dew"

deposition, the crystal dissolves, the organism is liberated and although the small amounts of extra moisture slightly impede the action of the gas they are readily sterilised.

Mayr (1961) and Perkins and Lloyd (1961) in advocating equilibrations at 95 per cent relative humidity, and at 55–60 per cent relative humidity, with various holding periods seem to be concerned (as is Phillips, 1961) with the state of hydration of the organisms themselves, although Perkins and Lloyd also mention the adsorption of moisture by solid surfaces. Equally, however, their findings can be explained by the solution of protecting substances as we have shown. Sodium chloride will pick up moisture at relative humidities above 75 per cent and we have observed that broth films become moist and sticky at lower humidities, about 50–60 per cent. Perkins and Lloyd also report that at 50–60 per cent relative humidity they were unable to sterilise *Cl. sporogenes* on a porous medium at levels greater than 10^5 organisms per 0·15 ml. but could sterilise the same organism at 10^6–10^7 organisms per 0·15 ml. when grown in a synthetic medium and dried on a solid surface. We have shown that porous media can be made to function as hard surfaces (and yield protected organisms) by exceeding their absorptive capacity with culture fluids containing organisms. It is thus apparent that the varying amounts of crystalloids, derived from different culture media, enclosing the organisms could explain these findings.

In stressing certain difficulties (many of which can be overcome) in the use of ethylene oxide, we do not wish to imply that ethylene oxide is other than a most valuable sterilising agent. Frequently it may be the only practicable method available, and in most cases in our own experience satisfactory sterilisation has been the rule and the "protected" organism the exception.

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The paper was presented by MR. ROYCE.

THE PREPARATION AND *IN VITRO* EVALUATION OF SOME MODIFIED ALUMINIUM HYDROXIDES AS GASTRIC ANTACIDS

By B. K. Davison and R. E. Schaffer

From the Research Department, Hardman and Holden Ltd., Manchester, 10

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The preparation and properties of some aluminium hydroxides modified by carbohydrates are described. *In vitro* evaluation of their antacid properties shows that they are quick acting products of high neutralising capacity and capable of prolonged buffering in the pH range 3 to 4. They compare well in a variety of tests with liquid aluminium hydroxide gel.

Liquid aluminium hydroxide gel has antacid activity which is both rapid and protracted in the desirable pH range 3 to 4. The dried gel on the other hand is slow in its antacid action, its activity is greatly diminished in the presence of pepsin and peptone, and further, there is a reduction in its rate of reaction with acids upon ageing.

The conditions used in preparation of the aluminium hydroxide influence the type of product formed (Lewis and Taylor, 1958); i.e. whether it is hydragillite, bayerite, boehmite or amorphous. When amorphous aluminium hydroxides prepared from aqueous solutions are dried to a critical water content, there is a rapid decrease in surface area upon storage (Harris and Sing, 1957). Such ageing does not occur in the dry state, but is dependent upon the presence of water vapour (Harris and Sing, 1957). However, aluminium hydroxides prepared from aluminium isopropoxide are much more resistant to ageing. The final surface area does not fall below 300 $m.^2/g.$, whereas those from aqueous solutions fall to about 60 $m.^2/g.$ (Harris and Sing, 1958). In addition, the low temperature nitrogen adsorption isotherms on products prepared from aluminium isopropoxide differ from those obtained on products from aqueous systems. The latter give a typical reversible S type curve indicating that there is no well defined pore structure, whilst those prepared from aluminium isopropoxide are more complicated, showing pronounced hysteresis loops characteristic of adsorption on a porous solid and resembling those obtained on "activated" alumina (Harris and Sing, 1960). It occurred to us that this type of aluminium hydroxide might be superior to dried aluminium hydroxide gel in antacid activity and in rapidity of action, and that it would not deteriorate so markedly upon storage. The use of carbohydrates as additives in the preparation of such products has been examined.

Experimental

Preparation of Products

Aluminium isopropoxide was freshly distilled before use.

Aluminium hydroxide. A solution of aluminium isopropoxide in an equal weight of xylene was heated under reflux with stirring and the theoretical quantity of water to convert the isopropoxide to hydroxide

was added dropwise over 1 hr. The precipitate was matured in the refluxing solution for a further 30 min., filtered off, washed with xylene and dried at 50°.

Aluminium hydroxide modified with carbohydrates. Aluminium isopropoxide (redistilled: 51 g., 102 g., or 204 g., depending on the additive-water ratio to be added) was added to isopropanol and the whole heated to reflux in an oil bath. The mixed reactants (additive and water) were then mixed with a further volume of isopropanol. The total isopropanol used to dilute the aluminium isopropoxide and reactants was always 400 ml. The mixed reactants were then added dropwise, with

TABLE I

MODIFIED ALUMINIUM HYDROXIDES

Additives	No. moles per mole aluminium isopropoxide	Weight g. aluminium isopropoxide	Weight additive g.	Weight H_2O g.	Yield g.
None	—	102	—	21	37
Sucrose	1/3	51	28·5	9	42
	1/6	51	14·25	9·75	31
	1/12	51	7·1	10·1	24
	1/24	102	7·1	20·6	42
	1/48	102	3·55	20·85	38
	1/96	102	1·77	20·95	36
Glucose	1/3	102	30	18·0	63
	1/6	102	15	19·5	52
	1/12	102	7·5	20·2	44
	1/24	102	3·8	20·6	42
Sorbitol	1/3	204	60·7	36	122
	1/6	204	30·3	39	92
	1/12	204	15·17	40·5	80
	1/24	204	7·6	41·2	80

stirring, to the aluminium isopropoxide over about 30 min.; the reaction mixture was heated a further 30 min. and, after cooling a little, the product was filtered off and air dried at 50° to constant weight.

With the higher additive-water ratios, where the amount of water was inadequate to dissolve the carbohydrate, the reactants were added in a slurry with isopropanol. All products were white powders. The glucose compounds, which were yellow when filtered off, dried to off-white powders. A modified aluminium hydroxide without carbohydrate, using water only as reactant, was also prepared.

A list of the modified aluminium hydroxides is given in Table I.

Evaluation of Products

Neutralising capacity. This was determined by the method of the British Pharmacopoeia 1958 for dried aluminium hydroxide gel.

Rapidity of action. A sample of the antacid (0·5 g.) was added, with stirring, to 50 ml. of 0·1N hydrochloric acid at 37°, and the pH values recorded after specific time intervals. Two or more determinations were made with each antacid.

Buffering capacity. A slurry of the compound under test was prepared by adding the finely powdered solid (1·0 g.) to distilled water (200 ml.).

The whole was maintained at 37° and stirred at a constant speed throughout the test. N Hydrochloric acid (1 ml.) was added and when the system had equilibrated the pH was recorded. Further 1 ml. quantities were added, and the pH recorded after each addition. The buffering capacity is defined as the number of ml. of N hydrochloric acid required to change the pH from 4 to 3, and was determined graphically.

Buffering action given by repeated doses. Three successive 0·5 g. samples were added at 15 min. intervals to 0·1N hydrochloric acid (50 ml.) at 37°. The pH values were recorded every 5 min., two or more determinations being made with each antacid.

TABLE II

THE EFFECT OF ADDITIVE UPON THE BUFFERING AND NEUTRALISING CAPACITY DETERMINED BY THE METHOD IN THE B.P. 1958 FOR DRIED ALUMINIUM HYDROXIDE GEL

Type of $Al(OH)_3$	pH after 10 min.	pH after 15 min.	pH after 20 min.	Ml. 0·1N NaOH	Neutralising capacity ml. 0·1N HCl
$Al(OH)_3$ ex xylene	1·54	1·57	1·59	99	102
Modified $Al(OH)_3$—					
No additive	1·9	2·4	3·5	24·2	251·6
Modified $Al(OH)_3$—					
Sucrose 1/3M	1·85	1·86	1·87	71·3	157·4
1/6	3·03	3·22	3·30	46·0	208·0
1/12	3·42	3·50	3·53	28·0	244·0
1/24	3·60	3·70	3·70	10·0	280·0
1/48	3·35	3·55	3·60	14·7	270·6
1/96	3·61	3·65	3·68	2·1	295·8
Modified $Al(OH)_3$—					
Glucose 1/3M	3·3	3·4	3·4	42·2	215·6
1/6	3·5	3·6	3·6	22·6	254·8
1/12	3·4	3·6	3·65	13·2	273·6
1/24	3·6	3·6	3·68	3·5	293·0
Modified $Al(OH)_3$—					
Sorbitol 1/3M	2·6	3·2	3·5	38·9	222·2
1/6	2·7	3·5	3·65	30	240
1/12	3·3	3·55	3·7	22·3	255·4
1/24	3·6	3·7	3·72	5·2	289·6
Dried Aluminium Hydroxide Gel B.P.	1·8	2·5	3·3	20·1	260

Buffering action in artificial gastric medium. The artificial gastric medium used was that of Brindle (1953) and consisted of 0·05N hydrochloric acid buffered with 0·15 per cent of pepsin, 0·15 per cent of peptone and 0·15 per cent of sodium chloride. The test method required the measurement at regular intervals of the pH of the artificial gastric juice at 37° from the time of adding the antacid (0·5 g.) until all the antacid had been consumed. Initially 150 ml. of artificial gastric juice was used, this had pH 1·45; 2 ml. of fresh juice were added every min. and after each 10 min. interval, 20 ml. of the total artificial gastric mixture were withdrawn and rejected.

Measurement of the specific surface area. The specific surface areas of the powders were determined by the air permeability method based on the resistance to the flow of air by a bed of powder packed under standardised conditions. The apparatus was that described by Rigden (1943) and Hughes (1959).

Results and Discussion

It is clear from the results that aluminium hydroxide precipitated from aluminium isopropoxide without additives offers no advantages over dried aluminium hydroxide gel B.P. as an antacid and that some modification of the composition is necessary if an improved antacid is to be

TABLE III

THE EFFECT OF ADDITIVE UPON THE BUFFERING CAPACITY, THE NEUTRALISING CAPACITY, THE SPECIFIC GRAVITY AND THE SPECIFIC SURFACE AREA

Type of $Al(OH)_3$	Buffering capacity ml. N HCl/g.	Neutralising capacity ml. 0·1N HCl/g.	Specific gravity	Surface area $m.^2/g.$
$Al(OH)_3$ ex xylene	7·1	102	2·1	6·2
Modified $Al(OH)_3$—				
No additive	13·6	251·6	2·0	12·0
Sucrose 1/3M	10·5	157·4	2·0	6·7
1/6	12·1	208·0	1·9	5·1
1/12	16·6	244·0	1·8	8·4
1/2	19·4	280·0	1·8	11·0
1/48	19·0	270·6	1·77	11·5
1/96	18·8	295·8	2·0	12·7
Glucose 1/3M	—	215	—	—
1/6	16	254	1·8	9·6
1/12	—	273	—	—
1/24	—	293	—	—
Sorbitol 1/3M	17	222·2	—	—
1/6	15	240·0	1·6	8·2
1/12	16	255·4	—	—
1/24	21	289·6	—	—
Dried Aluminium Hydroxide Gel B.P. ..	16·4	260	1·9	10·2

made from aluminium isopropoxide. The use of glucose and sucrose to suppress bayerite formation in aluminium hydroxides precipitated by the decomposition of sodium aluminate solution (Sato, 1960), made the use of such substances as modifying agents an obvious first choice. Additionally, not only do carbohydrates possess hydroxyl groups which can

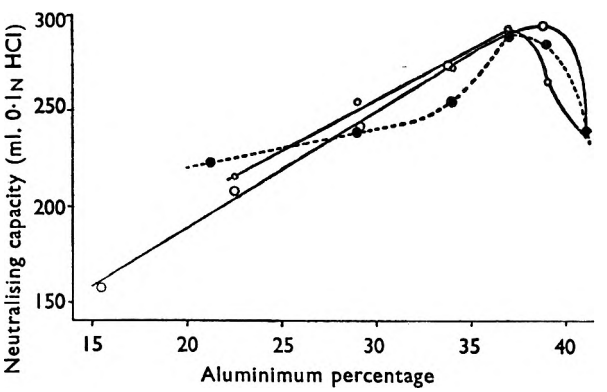

FIG. 1. The relation between neutralising capacity and aluminium percentage —○——○— = sucrose modified; —o——o— = glucose modified; -- ● --- ● -- = sorbitol modified.

replace the isopropoxy groups attached to aluminium in aluminium isopropoxide, but also they are non-toxic and can be assimilated by the body.

An ideal antacid should possess several properties; (i) it should be quick in its initial effect; (ii) the effect should be such that the pH of the gastric juices is not raised high enough to inhibit pepsin activity or to bring about acid rebound; (iii) it should have a high neutralising capacity and should maintain this effect over a prolonged period; (iv) it should be non-irritating to and should not be adsorbed by the alimentary tract; (v) it should not effervesce and cause flatulence, and (vi) it should be palatable.

A plot of the neutralising capacity (Table II) against aluminium percentage for each additive is given in Fig. 1. In every instance a curve is obtained with a maximum, showing that while a minimum amount of

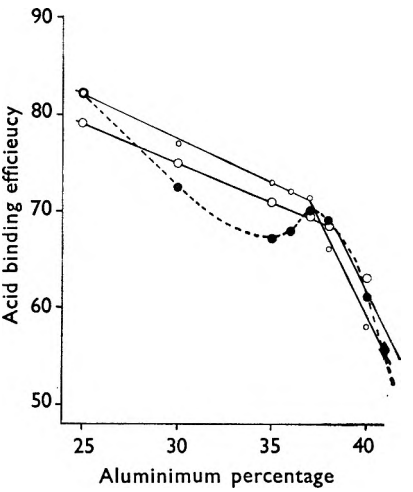

FIG. 2. The variation of acid binding efficiency with aluminium percentage. —○——○— = sucrose modified; —o——o— = glucose modified; -- ● --- ● -- = sorbitol modified.

additive is necessary to produce a rise in the neutralising capacity, larger quantities inevitably reduce this because they reduce the aluminium percentage. To eliminate the effect of this reduction in aluminium percentage for purposes of comparison, the "acid binding efficiency" has been calculated. For a compound of aluminium percentage X this can be expressed in the following way.

$$\text{Acid binding efficiency} = \frac{\text{neutralising capacity found}}{\frac{X}{100} \times 1110} \times 100 \text{ per cent}$$

since pure aluminium would have a neutralising capacity of $\frac{3 \times 10{,}000}{27}$ = 1,110 ml. 0·1N hydrochloric acid per gram. The acid binding efficiency plotted against aluminium percentage gives the curves shown in Fig. 2.

It is clear that the acid binding efficiency increases as the aluminium percentage falls: this happens with increasing amounts of additive. After the maximum neutralising capacity has been reached, the acid binding efficiency falls rapidly. The effect of the additives upon the speed of neutralisation can be seen from Fig. 3, where again decreasing aluminium percentage, i.e., increasing additive, brings about an increase in the speed

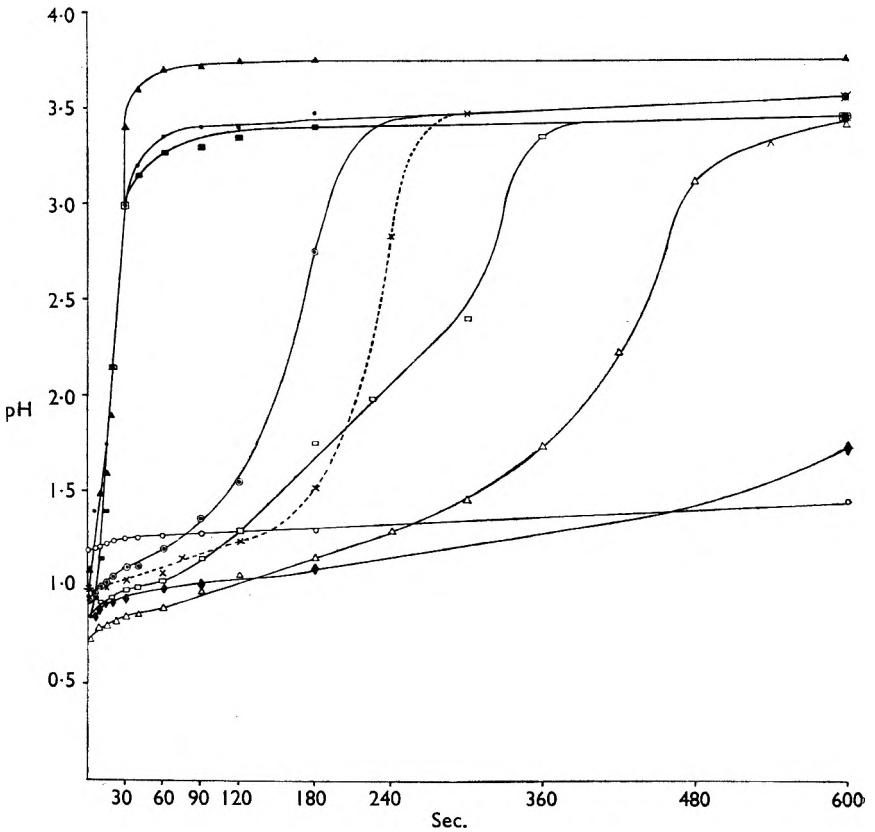

FIG. 3. The speed with which 0·5 g. quantities of the modified aluminium hydroxides react with 50 ml. of 0·1N hydrochloric acid at 37°. Modified $Al(OH)_3$ with: Sucrose 1/3 M —•—•—; 1/6 M —■—■—; 1/12 M —▲—▲—; 1/24 M —○—○—; 1/48 M —□—□—; 1/96 M —△—△—. No Additive —◆—◆—. $Al(OH)_3$ ex Xylene —○—○—. Dried Aluminium Hydroxide Gel B.P. - - - X - - - X - - -.

of action. The modified hydroxides are obviously superior to dried aluminium hydroxide gel in this respect; they raise the pH to 3 in 30 sec. as against minutes in the case of the latter. When buffering capacity is plotted against the aluminium percentage the curves shown in Fig. 4 result, and these are similar to those of Fig. 1. On repeated addition of sample to a standard quantity of acid, none of the hydroxides increased the pH of the solution above 4.

To obtain some idea of the behaviour of the modified hydroxides under conditions more closely resembling *in vivo* conditions, some have

been tested in artificial gastric juice. The results in Fig. 5 show that modified hydroxides are superior to dried aluminium hydroxide gel. They rapidly cause the pH to rise above 3, and maintain the pH in the physiologically desirable range of 3 to 4 for 30 min.

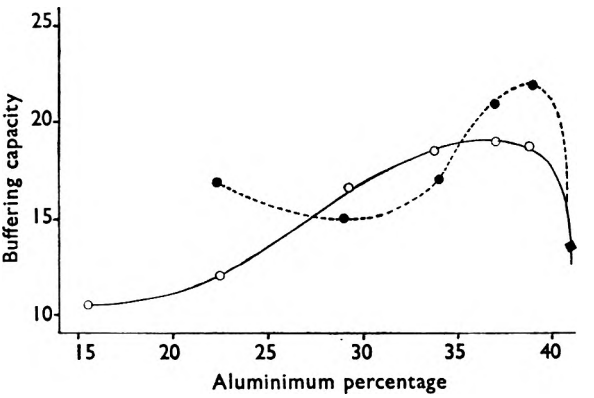

FIG. 4. The relation between buffering capacity and aluminium percentage. —○——○— = sucrose modified; - - - ● - - - ● - - - = sorbitol modified.

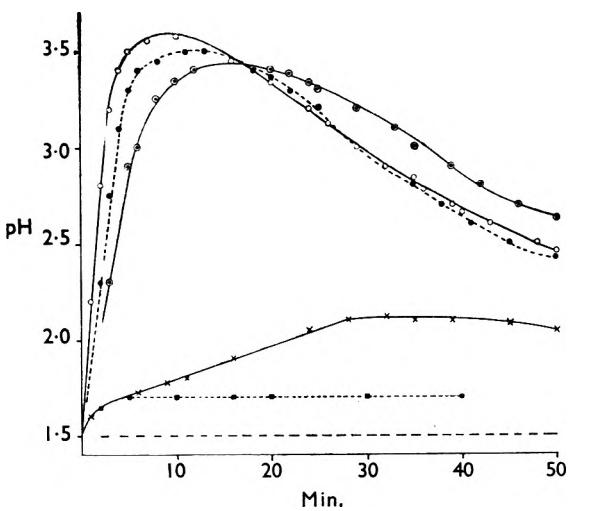

FIG. 5. The behaviour of the modified hydroxides in an artificial gastric medium. —○——○— = sucrose modified; —○——○— = glucose modified; - - ● - - - ● - - = sorbitol modified; - - ■ - - - ■ - - = (i) $Al(OH)_3$ no modifying agent added, and (ii) $Al(OH)_3$ ex xylene; —X——X— = dried aluminium hydroxide gel B.P.

The modified and unmodified hydroxides are believed to be amorphous, although it is not improbable that some boehmite may occur: this type of structure has been found when samples of amorphous aluminium hydroxide prepared from an aluminium alkoxide are allowed to age in ethanol (Bye and Robinson, 1961) and when aluminium hydroxide is

precipitated from acid solution. Aluminium isopropoxide in isopropanol as used in their preparation, is an acid solution. The alkoxo acid present:

$$Al(OR)_3 + R''OH \rightleftharpoons H^+ \left[\begin{matrix} Al(OR)_3 \\ \diagdown \\ OR'' \end{matrix} \right]^-$$

is titratable (Bersin and Meerwein, 1929).

No correlation of the surface area, as determined by the Rigden air permeability method, and the speed of action seems to exist. This method however gives but a relatively crude picture and more refined ideas of surface area are given by the method of low temperature gas adsorption, which may reveal some relation although this method has yet to be applied to our work.

All the products tested were palatable, showing no signs of astringency. Those products deemed suitable as antacids have been granulated and tableted without loss of activity. Samples stored for one year under ordinary atmospheric conditions have shown no loss in the speed of action and neutralising capacity tests.

Acknowledgments. The authors wish to thank Mr. M. H. Parkin, Mr. J. Latham and Mr. G. Shaw for technical assistance, and Mr. A. B. Newey for artificial gastric juice measurements.

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

The antacid effect was prolonged by increasing the dosage. The increased effects of the modified hydroxides were thought to be due to increased solubility of the products. A mixture of dried aluminium hydroxide gel, water and carbohydrate did not behave in the same way as the modified products. All the compounds had been tested in the same state of subdivision. Simple static tests can distinguish between products that are unsatisfactory as antacids and products that may be potentially useful, but dynamic tests are needed to simulate more closely gastric conditions. Using low temperature gas adsorption, the surface area of the products has been redetermined, and it was found to be the same for the modified products which reacted rapidly as for the slower-acting aluminium hydroxide from xylene.

NEUROMUSCULAR BLOCKING AGENTS

PART IX. SOME SHORT-ACTING LINEAR *NNN*-TRIS-ONIUM ESTERS

BY FIONA MACLEOD CAREY,* J. J. LEWIS,* J. B. STENLAKE† AND W. D. WILLIAMS†

*From the *Division of Experimental Pharmacology, Institute of Physiology, University of Glasgow and †The Department of Pharmacy, The Royal College of Science and Technology, Glasgow*

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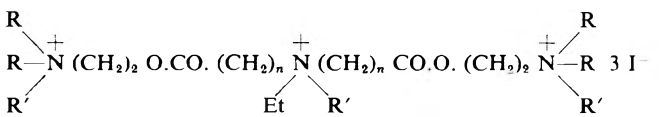
SUXAMETHONIUM (Fusco, Palazzo, Chiaverelli and Bovet, 1949), is the short-acting muscle relaxant most widely used in current clinical practice. This drug, however, has disadvantages; being a depolarising agent there is no satisfactory antidote and, owing to its initial stimulant action, it may cause post-operative muscular soreness (Churchill-Davidson, 1954; Foster, 1960; Morris and Dunn, 1957). More serious is the possibility of prolonged apnoea in patients deficient in pseudocholinesterase (Churchill-Davidson, 1959; Ottolenghi, 1959). For these reasons, attempts have been made to introduce short-acting non-depolarising muscle relaxants. Examples are 2,2′-dodecamethylene-bis-(*N*-dimethylpiperidinium), (Mantegazza, 1955), diohexadecanium bromide [Prodeconium; Prestonal; 3,14-dioxohexadecane-1,16-bis-(dimethylpropoxycarbonylmethylammonium) dibromide] (Frey, 1955; Griffith, Cullen and Welt, 1956), *p*-phenylene-bis-acetylcholine (Rosnati, 1957); 1,4-bis(4′-dimethylaminobutoxy)benzene dimethiodide (Bovet-Nitti, 1959), 2-diethylaminoethoxyethyl α-phenyl-α-1′-piperidylacetate dimethiodide (Cheymol, Guidicelli, Chabrier and Najer, 1959), γ-oxalolaudexium bromide (Brittain, Collier and D'Arcy, 1961) and the bis-onium tropeïne derivatives of Haining, Johnston and Smith (1960). None of the compounds introduced has yet succeeded in supplanting suxamethonium and there remains a need for a short-acting non-depolarising muscle relaxant (Foldes, 1957).

TABLE I

LINEAR TRIS-ONIUM ESTERS



Compound	*n*	R	R′	m.p.	Molecular formula	Found					Requires				
						C	H	I	N	Eq. (sap.)	C	H	I	N	Eq. (sap.)
201	2	Et	Et	174–176°	$C_{28}H_{56}I_3N_3O_4$	35·6	6·8	44·8	4·9	429	36·5	6·6	44·5	4·9	427·7
202	2	Et	Me	164–165°	$C_{23}H_{50}I_3N_3O_4$	33·8	6·5	46·5	5·1	414	33·95	6·2	46·8	5·2	406·7
203	2	Me	Me	166–167°	$C_{19}H_{42}I_3N_3O_4$	29·9	5·6	49·9	5·55	380	30·1	5·6	50·3	5·55	378·6
205	1	Et	Me	169–170°	$C_{21}H_{46}I_3N_3O_4$	32·4	6·3	48·6	5·3	397	32·1	5·9	48·5	5·35	392·7

In general, aliphatic linear ethonium esters are non-depolarising drugs of low potency. Potency may be increased by stepwise replacement of ethyl groups by methyl, although depolarising activity with its attendant

TABLE II

A QUALITATIVE COMPARISON OF THE NEUROMUSCULAR BLOCKING PROPERTIES OF COMPOUNDS 201, 202, 203 AND 205 WITH THOSE OF TUBOCURARINE AND SUXAMETHONIUM

	Effects upon blockade of the cat gastrocnemius-sciatic preparation of				Average duration of action at doses causing 40 to 60 per cent block (in min.)	
Compound	Neostigmine	Edrophonium	Ether	Tetanus	Cat	Hen
201	Antagonises	Antagonises	Prolongs	Transient decurarization	10	5
202	Antagonises	Antagonises	Prolongs		14	5
203	Potentiates	———	Antagonises	None	†	4
205			Prolongs		8	4
Tubocurarine	Antagonises	Antagonises	Prolongs	Transient decurarization	20	18
Suxamethonium	Potentiates	Potentiates		None	5	5

During block by 203 or suxamethonium, a tetanus was sustained and a contracture producing action was present in the frog and hen. The other compounds did not sustain tetanus and did not produce contracture.

† Block could only be produced in the neostigmine-treated cat by intra-arterial injection of the drug.

TABLE III

POTENCY AND TOXICITY

				Rabbit‡		Mouse			
Compound	Molecular weight	Cat* mg./kg.	Hen* mg./kg.	Single dose HD50 mg./kg.	LD50/HD50	i.p. PD50 mg./kg.	i.v. PD50 mg./kg.	LD50/PD50 i.v.	Frog† µg./ml.
201	855·4	1·7	2·3	14	>2·8	54	33	2·1	65
202	813·3	5·9	7·8	4·9	1·3	15	6·6	1·9	26
203	757·2	—	1·7	—	—	¶	¶	¶	—
205	785·3	>50	>50	—	—	—	—	—	—
Suxamethonium chloride	361·0	0·05	0·013	0·14	3·6	1·2	0·33	2·4	—
Tubocurarine chloride	785·7	0·12	0·35	—	—	0·38	0·07	3·0	1·6

* Dose causing 50 per cent inhibition of the gastrocnemius/sciatic preparation.
† Dose causing 50 per cent inhibition of acetylcholine contractions on rectus abdominis muscle.
‡ 202, slow injection gave a head drop dose in the rabbit of 8·6 mg./kg. while tubocurarine gave 0·31.
¶ Muscarine-like side effects prevented assay.

disadvantages then appears (Bovet, Bovet-Nitti, Guarino, Longo and Fusco, 1951; Ginzel, Klupp and Werner, 1952). Low potency, however, does not necessarily rule out the clinical trial of a muscle relaxant, provided that it is non-depolarising, has the required duration of effect, and has a satisfactory therapeutic index.

In Parts I–VIII of this series we have shown that linear polyethonium compounds, in which the onium groups are separated by five or six methylene groups or their equivalent, are tubocurarine-like. We have now synthesised the short series of linear tris-onium esters described in Table I.

The methods used for the pharmacological testing have been described previously by Edwards, Lewis, Stenlake and Zoha (1957; 1958). In Table II some qualitative actions of the four compounds are shown. Compounds 201, 202 and 205 are tubocurarine-like whilst the methonium derivative, compound 203, is a depolarising agent. Its contracture-producing action upon the hen gastrocnemius muscle is shown in Fig. 1.

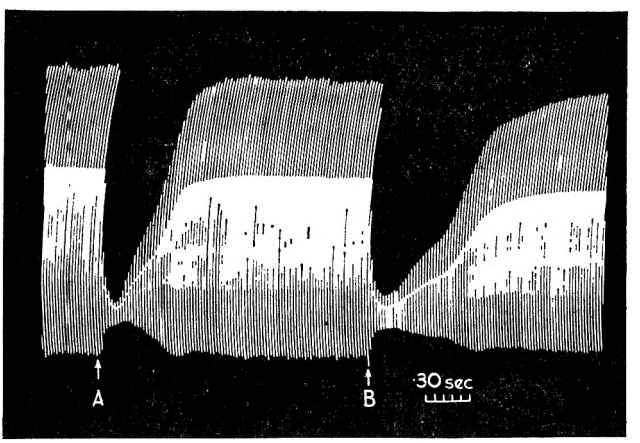


FIG. 1. Effects of 203 and suxamethonium on the gastrocnemius muscle-sciatic nerve preparation of the pentobarbitone-anaesthetised hen (2 kg.). A, 3·0 mg./kg. 203, i.v. causes an inhibition of contractions and the development of contracture.
Similar effects are seen with (B), 0·05 mg./kg. suxamethonium chloride, i.v.

In Table III provisional figures for the potencies and toxicities of compounds 201, 202, 203 and 205 are compared with those for tubocurarine and suxamethonium on the hen, cat, mouse, rabbit and frog.

Compounds 201 and 202 were much less potent than suxamethonium and tubocurarine, while the time of onset and their duration of effect was similar to that of suxamethonium (Table II). 205 was very weak indeed. In the hen, 203 had suxamethonium-like activity which was weaker and shorter in duration. It caused severe muscarine-like effects in the cat and mouse, produced a contracture of the frog rectus muscle and in the anaesthetised cat, induced a weak block. Equipotent muscle relaxant doses of 201, 202 and tubocurarine reduced the response of the nictitating membrane of the cat to electrical stimulation to about the same extent and both new compounds caused a brief depression of the arterial blood pressure of the anaesthetised cat. Some variations in potency between batches of compounds 201 and 202 have been observed, and the extent to which the rate of hydrolysis affects potency and duration of action of these esters is at present under investigation.

Acknowledgement. We wish to thank the National Research Development Corporation for financial support and Mrs Irene McKechnie for technical assistance.

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The paper was presented by MISS CAREY.

Short Communication

THE ANTI-INFLAMMATORY AND ANTIDIURETIC ACTIONS OF FRACTIONS OBTAINED FROM XANTHOGLABROL

By R. Best and R. S. H. Finney

From the Biology Department, College of Technology, Leicester

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During the preparation of potassium glycyrrhizinate by the method of Ruzicka and Leuenberger (1936) a yellow acidic material was isolated and named "Xanthoglabrol". In a preliminary report D'Arcy, Kellett and Somers (1957) showed that the sodium salts of this mixture had anti-inflammatory and antidiuretic activity in the rat. The separation of "Xanthoglabrol" into four fractions and an evaluation of their anti-inflammatory and antidiuretic properties is now described.

Fractionation of Xanthoglabrol. Partial evaporation of an ethereal solution of "xanthoglabrol" gave a yellowish white solid (Fraction IV). The remaining ether soluble material was recovered from the solvent and the mixture separated into three fractions on a column 11·5 × 3·25 cm. packed with aluminium oxide Grade I. The mixture (2 g.) was dissolved in 20 ml. of a solvent consisting of acetone (210 ml.), methanol (40 ml.) and 10 per cent aqueous sodium hydroxide (10 ml.) and placed upon the column. The same solvent was used for elution. Fraction I of the mixture was recovered from the first 260 ml. of the eluate, Fraction II from the following 325 ml. and Fraction III from the next 715 ml.

After evaporating each batch of eluate to dryness the residue was dissolved in water and the solution neutralised. The resulting precipitate was extracted with ether and recovered in the usual manner.

A total of 33 g. of crude material was chromatographed in this way yielding the following fractions: I, 7·6 g.; II, 13·2 g.; III, 9·0 g.

Anti-inflammatory activity. Cotton wool pellets were implanted subcutaneously into rats and after seven days, the pellets were removed, dried and weighed (Meier, Schuler and Desaulles, 1950). In the untreated animal the deposition of granulation tissue causes an increase in weight of the pellet. An anti-inflammatory compound depresses the production of granulation tissue. Four pellets were implanted in each rat in this way and at least five rats were used in each group.

Urine output and composition was investigated with groups of male rats starved for 18 hr. and then given 5 per cent of the body weight of distilled water by mouth as a loading-dose. Injection of the test material was made at this time, and the control animals treated with an equivalent volume of normal saline.

The urine output of each rat was noted at intervals over the next 5 hr. and the total 5 hr. urine sample of each rat was then analysed for sodium and potassium with a flame photometer. The urine volume and the sodium and potassium excretion of the test animals was compared with the controls.

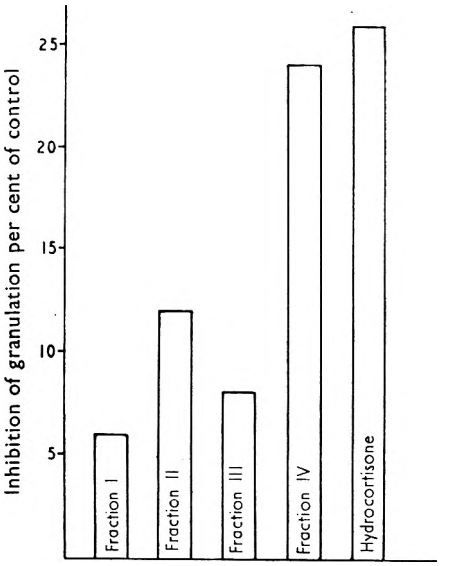

FIG. 1. The antiflammatory activity of "xanthoglabrol" fractions and hydrocortisone in the cotton-wool pellet test at 4 mg./rat/day.

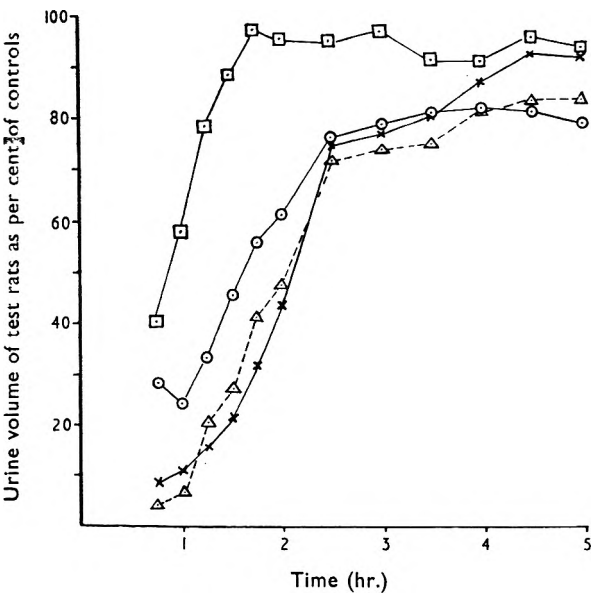

FIG. 2. The anti-diuretic action of "xanthoglabrol" fractions upon the rat at a dose of 2·5 mg./100 g. intraperitoneally.

○—○ Fraction I. △—△ Fraction II. □—□ Fraction III. X—X Fraction IV.

Each point is the mean of 10 observations.

RESULTS

Anti-inflammatory activity. All four fractions showed a linear relation between the anti-inflammatory activity and the logarithm of the dose.

As there was some daily variation in the results of the cotton pellet test, the relative activity of each fraction and of hydrocortisone was evaluated using groups of 15 rats, all fractions being examined over the same period. At a dose level of 4 mg./rat/day, Fraction IV had a greater activity than the other fractions, being similar in potency to hydrocortisone (Fig. 1).

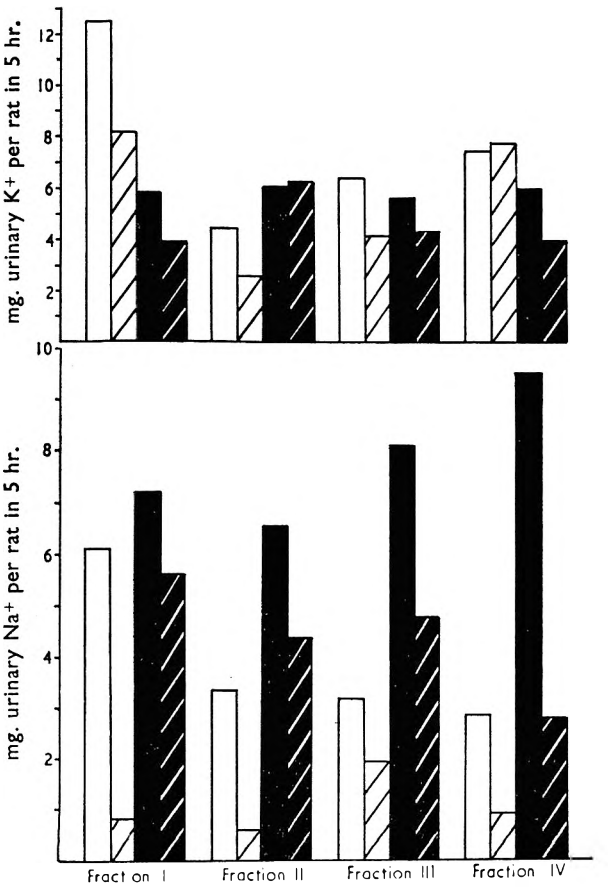

FIG. 3. Upper graph. The effect of "xanthoglabrol" fractions on urine potassium levels at a dose of 2·5 mg./100 g. sub-cutaneously to normal and adrenalectomised rats.

Lower graph. The effect of Xanthoglabrol fractions on urine sodium levels at a dose of 2·5 mg./100 g. sub-cutaneously to normal and adrenalectomised rats.

Unshaded column, normal control (10 rats).
Hatched column, normal test (10 rats).
Solid column, adrenalectomised control (16 rats).
Striped column, adrenalectomised test (16 rats).

Antidiuretic activity. With all fractions, an intraperitoneal injection of 2·5 mg./100 g. weight gave a strong antidiuretic effect (Fig. 2). This was accompanied by a reduction in the amount of sodium being excreted although the potassium excretion was only slightly affected. The same dose subcutaneously did not give the antidiuretic action, but examination of the urine showed a fall in sodium excretion, with a varying fall in potassium excretion.

Sodium excretion also fell in adrenalectomised rats similarly treated (Fig. 3).

These results are in sharp contrast to those obtained after deoxycorticosterone where a fall in sodium excretion occurred with an increase in urinary potassium levels (Marcus, Romanoff and Pincus, 1952), and it is suggested that the action of "Xanthoglabrol" upon urine electrolyte concentration is not mediated through the adrenal glands.

Acknowledgements. The authors wish to express their thanks to Professor E. E. Turner, F.R.S., Mr. D. E. M. Wotton, and Dr. S. Gottfried, of Biorex Laboratories Limited, London, E.C.1, for their advice, criticism and generous supply of materials, and Mr. H. S. Grainger of Westminster Hospital for the preparation of "xanthoglabrol".

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The paper was presented by MR. BEST.

THE DETERMINATION OF HEXACHLOROPHANE AND OTHER PHENOLS IN PHARMACEUTICAL PREPARATIONS BY A Δ E SPECTROPHOTOMETRIC METHOD

By D. A. Elvidge and B. Peutrell

From the Physical Assay Division, Standards Department, Boots Pure Drug Co. Ltd., Nottingham

Received May 23, 1961

The changes in spectral characteristics of phenolic compounds which occur with variations in pH have been used to develop a simple, rapid method for their determination in pharmaceutical preparations. Results compare favourably with those obtained by chemical analysis. The method is more specific than the chemical method.

In recent years derivatives of phenol such as hexachlorophane have found increasing use in the cosmetic field and this has necessitated more rapid and specific methods for their determination in complex formulations.

The method described in the U.S.P. XVI for the determination of hexachlorophane in Hexachlorophane Liquid Soap is based on that of Childs and Parks (1956). This depends on the fact that the spectrum of hexachlorophane at pH 3 is different from that at pH 8 whilst the soap base shows no such change. The maximum difference in extinction occurs at 312 mμ and the hexachlorophane content of the soap is determined by comparing the "difference" extinction (ΔE) of the sample with that of a standard solution of hexachlorophane.

The present work describes the application of the "difference" or ΔE method to preparations containing hexachlorophane, phenol, resorcinol, cresol, and methyl-*p*-hydroxybenzoate.

The U.S.P. XVI method for hexachlorophane recommends the use of two buffers, one (pH 1·4) obtained by diluting acetic acid (5 ml.) and hydrochloric acid (0·3 ml.) to 100 ml. with 90 per cent methanol; the other is obtained by adjusting 90 per cent methanol to pH 8·0 with sodium hydroxide. Batch to batch variation in the pH of the latter buffer solution led us to select tris-(hydroxymethyl)aminomethane (Bates and Bower, 1956). It has a good buffering action between pH 7 and 9, is fairly soluble in 90 per cent methanol, and is transparent to ultra-violet light. Using a 0·05M solution of tris-(hydroxymethyl)aminomethane in 90 per cent methanol it was found that to adjust the pH to 8·0, 50 ml. of 0·5N hydrochloric acid was required per litre of buffer solution.

Phenol, resorcinol and cresol were not ionised at pH 8·0 and a more alkaline solution was used for these compounds. This consisted of a 0·2M solution of potassium hydroxide in 90 per cent methanol.

Methyl *p*-hydroxybenzoate did not appear to show changes in spectral characteristics in the presence of large amounts of methanol but differences were observed in aqueous solutions. In phosphate buffer at pH 7·5 methyl *p*-hydroxybenzoate showed maximum absorption at 257 mμ and in 0·1N sodium hydroxide, at 296 mμ.

Experimental

Reagents

A. *Buffer solution pH* 8·0. Dissolve tris-(hydroxymethyl)aminomethane (6·07 g.) in methanol (900 ml.). Add 0·5N hydrochloric acid (50 ml.) and make up to 1 litre with water.

B. *Buffer solution pH* 1·4. Add glacial acetic acid (18 ml.) and concentrated hydrochloric acid (3 ml.) to methanol (900 ml.) and make up to 1 litre with water.

C. *Potassium hydroxide* 0·2 N *in methanol.*

D. *Buffer solution pH* 7·5. Dissolve potassium dihydrogen phosphate (22·2 g.) and dipotassium hydrogen phosphate (178 g.) in 1 litre of water.

E. 0·1N Sodium hydroxide.

F. Methanol.

TABLE I

SPECTROPHOTOMETRIC CHARACTERISTICS OF VARIOUS PHENOLS

Compound	Sample buffer	Blank buffer	Δ *E* (1 per cent, 1 cm.)	λ max. mμ
Hexachlorophane ..	A	B	144	312
Phenol	C	A	280	289
Resorcinol	C	A	305	291
Cresols B.P.	C	A	266	293
Methyl-*p*-hydroxybenzoate	E	D	1,290	296

Spectral Determinations on Pure Phenols

The "difference absorption spectra" of the alkaline solutions of hexachlorophane, phenol, resorcinol, cresol and methyl hydroxybenzoate were measured in a 1 cm. cuvette from 220 to 350 mμ using the more acid solution of the same strength of phenol in the reference cuvette. With a series of varying concentrations it was found that Beer's Law was obeyed in all instances. The appropriate buffer, Δ*E* (1 per cent, 1 cm.) values and wavelength criteria are given in Table I.

Some preparations contained salicylic acid or dichlorophane in addition to hexachlorophane. The absorption spectra of these two compounds were measured in the buffer solutions used for hexachlorophane. It was found that at the wavelength where hexachlorophane showed a maximum value of Δ*E* (1 per cent, 1 cm.) both salicylic acid and dichlorophane also showed changes in *E* (1 per cent, 1 cm.) with pH. It was found, however, that at 257·5 and 263 mμ respectively the extinctions of salicylic acid and dichlorophane were independent of changes in pH. At these wavelengths the corresponding Δ*E* (1 per cent, 1 cm.) values for hexachlorophane were 111 and 69·6, and the results in Table III for the samples containing salicylic acid and dichlorophane were obtained using these values.

Recovery Experiments

To check the efficiency of the "difference" method we examined where possible the effects of the ingredients of the base of each preparation.

Known amounts of the phenolic compound concerned were added to the appropriate ingredients and where these were not available, known amounts of the phenols were added to the samples themselves. The range of recoveries was 97 to 104 per cent (see Table II).

Methods of Extraction

Three general methods were used.

(*a*) Direct dilution or extraction with the appropriate buffer solution.

(*b*) Extraction with chloroform.

(*c*) Extraction with light petroleum (40–60°)-methanol mixtures.

TABLE II

RECOVERY OF PHENOLS IN VARIOUS PREPARATIONS

Sample	Extraction		Recovery of added phenol per cent
	Procedure‡	Reagent‡	
HEXACHLOROPHANE			
Shampoo	(*a*)	A	98·0
Soap (1)	(*a*)	A	103·0
Soap (2)	(*a*)	A	103·0
Deodorant stick (1)	(*a*)	A	104·0
Deodorant stick (2)	(*a*)	A	104·0
Talcum powder (1)	(*b*)	A	99·7
Talcum powder (2)	(*b*)	A	99·0
Talcum Powder (3)	(*b*)	A	100·0
Pre-shave lotion	(*a*)	A	99·3
Shaving lather	(*a*)	A	98·8
Veterinary shampoo	(*a*)	A	99·3
Veterinary cream	(*a*)	A	97·0
Foot talc*	(*b*)	A	100·0
Hair lotion†	(*a*)	A	101·5
PHENOL			
Compound solution of Sodium Phenate B.P.C. 1949	(*a*)	F	101·0
Glycerin of Phenol B.P.	(*a*)	F	99·0, 97·0
Calamine Lotion B.P.	(*a*)	C	100·0
Zinc Oxide Cream	(*a*)	C	101·0
RESORCINOL			
Resorcinol Ointment B.P.C. 1949	(*c*)	F	101·0
Compound Resorcinol Ointment B.P.C.	(*c*)	F	99·4
CRESOLS B.P.			
Veterinary shampoo	(*a*)	F	98·0
Lysol B.P.	(*a*)	F	100·0
METHYL HYDROXYBENZOATE			
A complex proprietary liquid preparation	(*c*)	F	99·4

* Contains dichlorophane. The hexachlorophane content was calculated using the ΔE (1 per cent, 1 cm. value at 263 mμ.

† Contains salicylic acid. The hexachlorophane content was calculated using the ΔE (1 per cent, 1 cm. value at 257·5 mμ.

‡ Refer to text for description.

With one exception, method (*a*) was applied to all liquid preparations. Preparation of sample: Weigh or pipette a suitable amount into a 100 ml. stoppered conical flask, add about 50 ml. of buffer solution appropriate to the phenol being examined (Table I). Shake or warm until the sample is completely dispersed then filter under vacuum through a Whatman No. 42 filter paper. Wash the conical flask and filter paper with three portions, each of 10 ml., of buffer solution and transfer the filtrate quantitatively to a 100 ml. graduated flask. Make up to volume with buffer solution.

Method (*b*) was applicable to samples in powder form. Preparation of sample: Weigh a suitable amount into a sintered glass funnel (No. 3 porosity). Extract the powder with five successive portions, each of about 20 ml., of chloroform, drawing each extract through the sinter with gentle suction and collecting the extract in a Buchner flask. Remove the chloroform on a steam-bath taking care to avoid volatilisation of the phenol and dissolve the residue in a suitable volume of the appropriate buffer solution.

Method (*c*) was found suitable for ointments. Preparation of sample: Weigh a suitable amount into a small beaker, add 20 ml. of light petroleum

TABLE III

PHENOL CONTENT OF VARIOUS PREPARATIONS

Sample	Phenol expected per cent	Phenol found by proposed method per cent	Phenol found by chemical method per cent
HEXACHLOROPHANE			
Shampoo	1·0	1·02, 1·04, 1·02, 1·02	—
Soap (1)	1·0	1·16, 1·17	—
Soap (2)	1·0	1·04, 1·03, 1·03	—
Deodorant stick (1)	0·25	0·29, 0·30	—
Deodorant stick (2)	0·25	0·31, 0·32, 0·32	—
Talcum powder (1)	0·25	0·25, 0·25	—
Talcum powder (2)	0·25	0·24, 0·25	—
Talcum powder (3)	0·20	0·20, 0·20	—
Pre-shave lotion	0·20	0·20, 0·21	—
Shaving lather	0·20	0·20, 0·20	—
Veterinary shampoo	0·50	0·50, 0·50	—
Veterinary cream	0·50	0·48, 0·49	—
Foot talc.	0·50	0·51, 0·51	—
Hair lotion	0·20	0·20, 0·20	—
PHENOL			
Compound Solution of Sodium Phenate B.P.C. 1949	2·8–3·4	3·31, 3·31	3·12
Glycerin of Phenol B.P.	15·0–16·5	16·7, 16·9	15·8, 15·8
Calamine Lotion B.P.	0·39–0·47	0·46, 0·45	0·46
Zinc Oxide Cream	0·36–0·41	0·39, 0·39	0·40
RESORCINOL			
Resorcinol Ointment B.P.C. 1949	12·5	12·6, 12·6	12·2
Compound Resorcinol Ointment B.P.C.	4·0	4·07, 4·07	4·34
CRESOLS			
Veterinary shampoo	4·0	3·9, 3·9	4·10
Lysol B.P.	47–53	51·0, 51·0	51·0
METHYL HYDROXYBENZOATE			
A complex proprietary liquid preparation	0·12	0·11, 0·12	—

and disperse the sample as far as possible with a glass rod. Allow to settle and decant the supernatant liquid into a 150 ml. separator. Wash the beaker successively with the following solutions, transferring each in turn to the separator. Three portions, each of 20 ml., of light petroleum-methanol (1 : 1). Two portions, each of 10 ml., of methanol. One portion of 10 ml. of light petroleum-methanol (1 : 1). Finally add 15 ml. of water to the separator, stopper and shake gently for 1 min. Allow the layers to separate, filter the lower layer through a small plug of cotton wool into a 100 ml. graduated flask, wash the light petroleum layer in the separator with 10 ml. of methanol-water (1 : 1) and add these washings to the graduated flask through the cotton wool plug. Make up to volume with methanol.

With the complex proprietary liquid preparation examined, the methyl *p*-hydroxybenzoate was extracted directly with ether, the ether removed by evaporation and the residue dissolved in methanol. Subsequent dilutions were made in phosphate buffer pH 7·5 and 0·1N sodium hydroxide, the spectrophotometric measurements being made as rapidly as possible to avoid possible hydrolysis effects.

Spectrophotometric Determination

Dilute a suitable aliquot of the extracted phenolic compound with the "sample" buffer solution and a second aliquot in the appropriate "blank" buffer solution (listed in Table I). It is important that the concentration

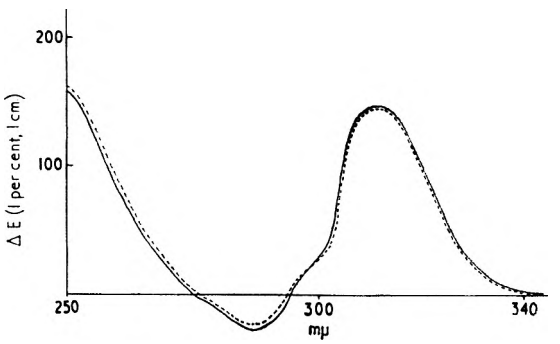

FIG. 1. △ *E* curve for pure hexachlorophane. (——) compared with △ *E* curve for hexachlorophane in a talcum powder. (– – –).

of the phenolic compound is the same in both "sample" and "blank" buffer solutions. For all the samples examined, the final dilution with the appropriate buffer solution was at least tenfold. Under these conditions the pH value of the final solution was found to be within 0·1 units of that of the diluent buffer.

Measure the extinction of the more alkaline solution in a 1 cm. cuvette at the appropriate wavelength (see Table I) using the more acid solution in the reference cuvette. Calculate the content of phenolic compound in the sample using the ΔE (1 per cent, 1 cm.) value found for the pure phenol in the same pair of buffer solutions. The figures quoted in Table I are intended only as a guide.

RESULTS

Details of extraction procedure, dilutions and recoveries obtained are shown in Table II. Results on the samples examined are given in Table III. It will be seen that quantitative determinations of a variety of phenols in a wide range of cosmetics and pharmaceutical preparations have been achieved. Comparison with chemical analysis, when available, shows good agreement. By obtaining the complete difference spectrum of the extracted phenolic compound a qualitative identification is also

achieved, as shown in the example in Fig. 1 for the hexachlorophane content of a talcum powder.

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The paper was presented by MR. ELVIDGE.

THE SPECTROPHOTOMETRIC DETERMINATION OF THALIDOMIDE IN BODY FLUIDS

By J. N. Green and B. C. Benson

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

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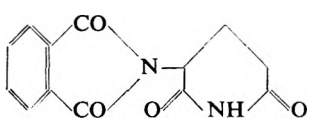
An ultra-violet spectrophotometric method is described for the determination of thalidomide in blood, plasma or urine. Thalidomide is extracted from the sample with chloroform, and the drop in extinction measured when solutions are treated with alkali. The method has been used to determine the amount of drug present in animal and human blood.

The ultra-violet absorption spectrum of thalidomide (I) in 0·1N hydrochloric acid exhibits maxima at 220 mμ and 299·5 mμ (Fig. 1) and the concentration of this material may be measured with high sensitivity using the first of these two peaks where E (1 per cent, 1 cm.) = 1,950. In absolute ethanol the spectrum shows but minor changes, $\lambda_{max.}$ appearing at 218 mμ (E, 1 per cent, 1 cm. = 2,010), and 292·5 mμ. When solutions of thalidomide in either of these solvents are treated with alkali there results a decrease in extinction at the peak wavelengths. This decrease is proportional to the concentration of thalidomide present, and provides a basis for an assay of this substance.



(I)

During this work Beckmann and Kampf (1961) reported a practically identical method for estimating thalidomide in body fluids. Our results discussed below confirm the work of the German authors and underline the usefulness of such a procedure for the estimation of this substance. It is suggested by Beckmann and Kampf that treatment of thalidomide with alkali results in the hydrolysis of the phthalimide ring to an *N*-substituted phthalamic acid. We are in agreement with this suggestion.

Experimental

All spectral determinations were made using a Unicam SP500 spectrophotometer.

Extraction of thalidomide from aqueous solutions in the pH range 1–7·4 may be accomplished with chloroform, and this solvent is also suitable for extracting thalidomide from body fluids. Basic impurities may be removed from such extracts by washing with dilute acid, but attempts to remove acidic impurities with dilute alkali lead to destruction of the thalidomide.

Samples of blood, plasma or urine (5–10 ml.) were extracted with 50·0 ml. of chloroform in a stoppered flask by shaking vigorously for approximately 5 min. The solvent extract was run through a Whatman No. 41 filter paper and washed once by shaking with 5–10 ml. of 0·1N hydrochloric acid. The acid wash was discarded and the solvent was again filtered through Whatman No. 41 paper; 40 ml. of the clear filtrate was evaporated to dryness in a vacuum desiccator.

The dry residue was dissolved in 0·1N hydrochloric acid at 60–65° and filtered through an acid washed Whatman No. 1 paper, then made up to a final volume of 8·0 ml. The extinction (E_1) of 3·0 ml. of this

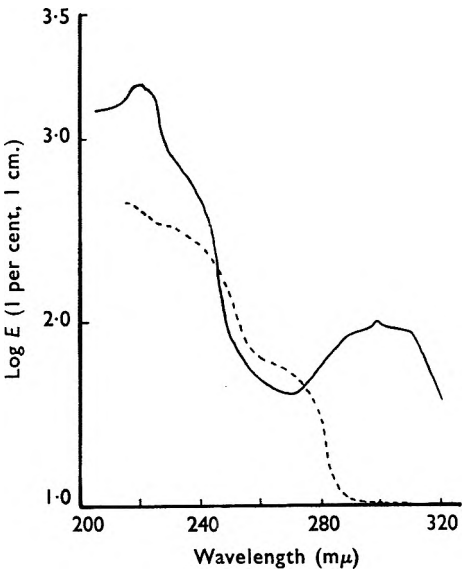


FIG. 1. The ultra-violet absorption curve of thalidomide in 0·1 N hydrochloric acid. ——— Thalidomide in acid solution. – – – – – After treatment with alkali.

solution was measured at 220 mμ against the 0·1N hydrochloric acid used as solvent (in 1 cm. cells). To both solution and solvent cells, 0·50 ml. of N sodium hydroxide was added. The solutions were stirred, and after 15 min. the extinction (E_2) at 220 mμ was again determined. This procedure was repeated on a second 3 ml. aliquot of the test solution, and the mean of the two values for E_1–E_2 gave ΔE_T for the test solution. A blank determination was carried out in an identical manner on blood containing no thalidomide to give ΔE_B for the blank solution. Then $\Delta E_T - \Delta E_B = \Delta E$, the extinction difference due to thalidomide. The weight (W) of thalidomide which this represented was read from a standard curve, and the concentration (C) of the drug in the test sample then = W × 8 × 50/40 × volume of sample in ml. Owing to the low solubility of thalidomide in water the standard curve was obtained as follows. A saturated aqueous solution of thalidomide was prepared. To 9 ml. of this solution was added 1 ml. of dimethylformamide, and the extinction

of this solution at 299 mμ was determined. For a 10 per cent w/v solution in dimethylformamide the E (1 per cent, 1 cm.) at 299 mμ = 93·2, and this value was used to calculate the concentration of thalidomide in

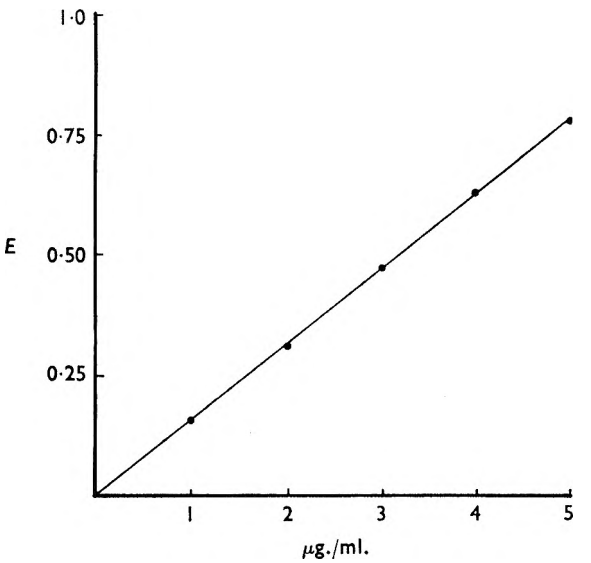


FIG. 2. Calibration curve for thalidomide in 0·1 N hydrochloric acid. $E = E_1 - E_2$ at 220 mμ.

the saturated aqueous solution. By stepwise dilution of the saturated aqueous solution with 0·1N hydrochloric acid, concentrations ranging from 1–5 μg./ml. were obtained and the standard curve constructed from these (Fig. 2).

As an alternative assay procedure, the dry residue was dissolved in 8·0 ml. of absolute ethanol and centrifuged, ΔE values were determined on aliquot samples of this solution at 218 mμ essentially as described above, using 0·2N potassium hydroxide as alkali. Solutions for the

TABLE I

RECOVERY OF KNOWN AMOUNTS OF THALIDOMIDE

No. of experiments	5 ml. sample	Thalidomide added μg.	Solvent	Calculated recovery and standard error
1*	Water	5	Acid	5
13*	Whole blood	5	Acid	4·5 ± 0·32
3*	Water	8·5	Acid	8·33
3	Plasma	10	Acid	9·72
11	Whole blood	10	Acid	9·65 ± 0·5
12	Whole blood	20	Acid	19·22 ± 1·57
10	Whole blood	50	Acid	40·5 ± 3·05
2	Whole blood	100	Acid	68·35
11	Whole blood	10	Ethanol	9·69 ± 0·56
12	Whole blood	20	Ethanol	19·59 ± 1·27
8	Whole blood	50	Ethanol	46·23 ± 2·16
4	Whole blood	100	Ethanol	93·1 ± 5·5
4	Urine	20	Ethanol	18·6 ± 0·95

* In these experiments the dry residue was dissolved in 5 ml. of acid.

construction of the standard curve were obtained by dissolving a known weight of thalidomide in absolute ethanol, and then diluting to give the desired concentration range.

Results and Discussion

The accuracy of the analytical procedure was checked by adding known amounts of thalidomide to 5 ml. aliquots of blood, plasma or urine and then carrying out the assay described. Details are given in Table I.

These results show that with thalidomide blood levels up to 4 μg./ml. the standard error of the assay procedure is $\pm$ 7·5 per cent with a

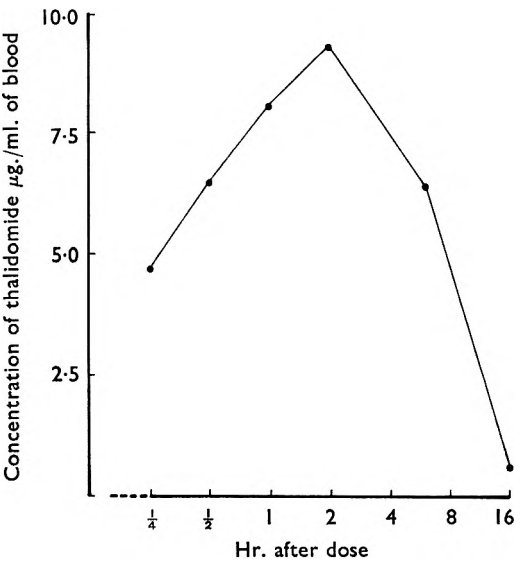


Fig. 3. The concentration of thalidomide in mouse blood after oral dosing. (10 mg./20 g. mouse).

recovery efficiency of over 90 per cent irrespective of whether ethanol or 0·1N hydrochloric acid is used. At blood concentrations above 10 μg./ml. better results are obtained with ethanol, as in the alternative procedure.

Absorption measurements from 180–250 mμ are liable to much error due among other effects to stray light. The fact that Beer's law is followed (Fig. 2) taken with the satisfactory recovery of thalidomide from biological fluids, however (Table I), demonstrates that the assay is satisfactory for the purposes stated.

Potassium hydroxide of the strength used in the alternative assay with ethanol has been reported as acting as a cut-off filter just below 218 mμ, and when the extinction value of the ethanol:0·2N potassium hydroxide mixture was measured against air at 218 mμ, a figure of 1·65 was obtained. In spite of this fact, that approximately 97 per cent of the incident light was absorbed by this solvent mixture, the method

yielded satisfactory results (Table I). Extinction measurements made on an ethanol:0·1N potassium hydroxide mixture gave a value of 1·0, whilst the 0·1N acid:N sodium hydroxide mixture measured at 220 mμ gave a reading of 0·220. These extinction values suggest that the use of a potassium hydroxide solution weaker than 0·2N would be advisable.

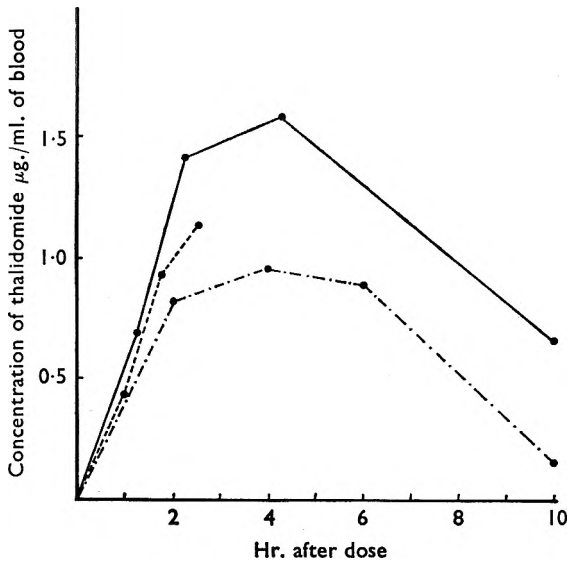


FIG. 4. The concentration of thalidomide in the blood of human volunteers; 3 volunteers, each given a 1 × 150 mg. tablet of thalidomide.

Blood determinations in man and experimental animals have been carried out by this method. In mice the curve shown in Fig. 3 resulted. Blood levels of the drug in three human volunteers were as shown in Fig. 4.

Acknowledgement. The authors wish to express their thanks to Dr. C. W. M. Wilson and Dr. K. Martindale for supplying the human blood samples on which the thalidomide determinations were carried out.

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The paper was presented by DR. GREEN.

THE EFFECT OF A SULPHATED POLYSACCHARIDE UPON THE DIFFUSION OF PEPSIN THROUGH MUCIN

By W. Anderson

From the Evans Medical Research Laboratories, Liverpool, 24

Received May 18, 1961

Degraded carrageenan, a sulphated polysaccharide, is shown to hinder the diffusion of pepsin through mucin *in vitro* when it is mixed with the mucin before the application of the pepsin solution. It is suggested that this property is part of the mechanism by which degraded carrageenan protects experimental animals from histamine ulceration.

Experimental animals can be protected from histamine-induced peptic ulceration by giving them certain sulphated polysaccharides orally (Levey and Sheinfeld, 1954; Anderson and Watt, 1959). Sulphated polysaccharides diminish peptic activity *in vitro* mainly by reacting with and protecting the substrate, rather than by inactivating the enzyme (Anderson, 1961). Their mode of action *in vivo* is unlikely to be by direct antipeptic activity. A partial explanation may be that the polysaccharides protect the mucosa from the gastric juice by reacting with mucoprotein to give an increase in strength of its mucinous properties, thereby causing an increased resistance to the diffusion of gastric secretion through the mucoprotein to the mucosa. The work now reported was designed to demonstrate this hypothesis *in vitro*.

Experimental

Materials and Methods

Sulphated polysaccharide. Degraded carrageenan* was used (Anderson, 1961). A 10 per cent solution gave a pH of 6·8.

Mucin. 10 g. gastric mucin (Armour Laboratories) intimately mixed with 100 ml. water, was centrifuged to deposit gross insoluble matter, and the slightly opalescent viscous supernatant solution was used. This was called 10 per cent mucin; it contained 0·4 per cent nitrogen and had a pH of about 5·5. In the experiments it was diluted either with equal parts of water to give 5 per cent mucin, or with equal parts of a 10 per cent aqueous solution of degraded carrageenan to give 5 per cent mucin containing 5 per cent degraded carrageenan. The pH of the mucin and of the degraded carrageenan solutions were not adjusted, except where indicated.

Pepsin solution. Granular 1 : 10,000 pepsin at a concentration of 20 mg./ml., adjusted to pH 1·6 with HCl.

Acid solution. Solution of HCl in water at pH 1·6.

Haemoglobin solution. 0·5 per cent bovine haemoglobin enzyme substrate powder (Armour Laboratories) in HCl solution adjusted to pH 1·6.

Method. A double-end cell was used to simulate idealised conditions in the gastrointestinal tract.

*Ebimar (Evans Medical Ltd.).

The cell was constructed from a tube which was divided in two by a porosity 4 fritted disc 10 mm. in diameter and 2 mm. thick, which was fused into the centre of the tube. The ends of the tube were of a length such that when the rubber stoppers were inserted to a given mark the volume in each limb was 5 ml. The size disc used was found to be the most suitable because it allowed reasonable diffusion rates without applying pressure, while not allowing bulk streaming of the liquids. With simple diffusion experiments using pepsin solution and discs of the same nominal porosity, it was found that each tube had a specific diffusion rate, which was related approximately to the suction-filtration rate. Tubes having the same resistance to flow characteristics were grouped in threes. Each group of cells was used in turn for each experiment (that is: no mucin, mucin, mucin plus degraded carrageenan). Before use, and after cleaning, the tubes were washed with acid solution by suction, the disc being left saturated with acid solution. The inner walls of the tube limbs were dried and 5 ml. of haemoglobin solution at 35° placed in the lower limb. A hypodermic needle inserted through the rubber stopper allowed it to be inserted to the mark without including air and without forcing the solution into the fritted disc. The needle was then removed. Into the upper limb was placed: 0·5 ml. of 5 per cent mucin; or 0·5 ml. of 5 per cent mucin containing 5 per cent degraded carrageenan; or 0·5 ml. water, to give a layer 7 mm. deep. The pepsin solution was layered on to this so that, apart from the water samples, a distinct interface was formed. All solutions were at 35°. After filling, the tubes were stoppered and supported vertically in a water bath controlled at 35°. After 6 hr. incubation the haemoglobin solution was poured into 10 ml. of 10 per cent trichloroacetic acid. The filtrate from this was taken to measure the amount of peptic digestion using Folin-Ciocalteu's reagent as described by Anderson (1961); the optical densities of the solutions indicating the extent of peptic digestion of the haemoglobin in the lower limb, this in turn being a measure of the pepsin gaining access to, and being incubated with, the substrate within 6 hr.

TABLE I

THE DIGESTION (INDICATED BY OPTICAL DENSITY × 100) OF HAEMOGLOBIN IN 6 HR. BY PEPSIN IN THE ABSENCE OF MUCIN; AFTER DIFFUSION THROUGH MUCIN; AND AFTER DIFFUSION THROUGH MUCIN PLUS DEGRADED CARRAGEENAN

Cell number	No mucin	Mucin	Mucin plus degraded carrageenan
1	46·5	7·5	10·0
5	38·0	8·5	8·0
6	32·5	5·0	9·5
17	47·5	16·5	4·5
8	42·0	27·0	8·5
9	56·5	29·5	8·0
2	37·5	12·0	7·0
3	21·0	24·0	12·0
13	84·0	19·5	18·0

Significance of difference between the means of columns two and three taken alone: $t = 2{\cdot}19$; $P = 0{\cdot}02$–$0{\cdot}05$

Results

The results are given in Table I.

Effect of the Sulphated Polysaccharide in the Pepsin Solution

Similar experiments were made where different amounts of degraded carrageenan were dissolved in the pepsin solution. More than 1 per cent of degraded carrageenan gave rise to difficulty in layering the solution onto the mucin because of alteration in density. Nevertheless, even 1 per cent degraded carrageenan in the pepsin layer did not retard diffusion of pepsin through the mucin under these conditions, as compared with the diffusion when degraded carrageenan was absent from the pepsin solution. The results of the mean digestions (indicated by optical density $\times$ 100) of haemoglobin in 6 hr. are: by pepsin in the absence of mucin, 49·8; after diffusion through mucin, 17·8; and after diffusion through mucin, the pepsin first having been mixed with degraded carrageenan, 20·7.

Diffusion of Carrageenan

Some degraded carrageenan diffused from the mucin through the fritted disc, but the quantity diffusing in 6 hr. was small (ca. 0·2 mg.) and would not be expected to account for the diminished digestion seen. This experiment was made by replacing the haemoglobin solution with acid solution and estimating the degraded carrageenan found therein after the 6 hr., with toluidine blue. The results are: mg. degraded carrageenan found in the lower limb in 1 hr. 0; 3 hr. 0·04; 5 hr. 0·2; 6 hr. 0·2.

Diffusion of Acid

The diffusion of acid from the pepsin layer through the mucin could be seen as a descending opacity due to precipitation in the mucin layer and this was usually complete in the first half hour. When degraded carrageenan was present in the mucin the acid did not do this to the same extent but a thin (about 1–2 mm.) opaque pellicle formed at the interface. This began to form as soon as the acid or acid-pepsin was layered and appeared to impart a cohesiveness to the mucin surface which was stronger as a result, and obviously withstood the occasional impact during layering much better than when degraded carrageenan was absent.

Depth of Mucin Layer

Layers of 0·2 ml. (giving 3 mm.) and 1 ml. (giving 13 mm.) mucin were also tried. 0·2 ml. gave a layer so shallow that floating of the pepsin solution was a hazardous and tiresome operation, whereas the 1 ml. layer gave results similar to those with 0·5 ml.

Discussion

The results show that a sulphated polysaccharide added to mucin hinders the passage of pepsin through the mucin. They are comparative and the conditions of the experiment are admittedly ideal, a steadier state being substituted for the dynamic state existing in natural conditions.

The concentrations and quantities of materials have been chosen for convenience and only in their disposition do they resemble the natural order inasmuch as the acid gastric juice bathes a mucinous layer which is supported on closely-knit structures which separate it from the mucosa. The haemoglobin solution is a convenient experimental substitute for denatured digestible tissue. The mucin was used without adjustment of pH which was 5·5 and therefore probably close to its natural reaction. It was thought that if degraded carrageenan was mixed with it at this pH, reaction between them would mostly occur as the acid and, more slowly, the pepsin, diffused through the layer giving a greater opportunity for impeding the progress of the pepsin.

The concentrations of the mucin, pepsin and degraded carrageenan were chosen principally on a density basis to avoid mixing or inversion. Similarly in the experiments where the sulphated polysaccharide was present in the pepsin layer its concentration could not have been increased greatly without causing the same difficulty. The depth of the mucin layer was also of importance, and this is believed to be a natural factor in its protective function. It was observed that layering of the pepsin solution without deformation of the interface was markedly easier on mucin containing sulphated polysaccharide, and this was thought to be due to the rapid formation on the surface of the mucin of a "pellicle" which could clearly be seen when it expanded to about 1–2 mm. in depth during the experiment.

The difference between the effect of mucin and that of mucin plus degraded carrageenan can be exaggerated by prolonging the incubation period. Although periods longer than 6 hr. become unrealistic, it was clear in these experiments that the passage of pepsin through, and from, mucin containing sulphated polysaccharide is very severely curtailed.

This process of restricting the access of pepsin from mucin to the mucosa is suggested as a contributory factor in the ulcer-preventing activity of sulphated polysaccharides which has been reported in the laboratory animal. It is now known that these substances also diminish peptic activity by protecting the substrate or digestible tissue in the presence of acid. It is not intended to imply that these two experimental observations explain the dramatic protection of the experimental animals from the ulcerative effects of large volumes of strong gastric juice secreted under histamine or stress stimulation, but they must be aspects of a many-sided action on the elements of gastric secretion.

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The paper was presented by THE AUTHOR.

ION-EXCHANGE CHROMATOGRAPHY ON ALGINIC ACID OF CERTAIN B-GROUP VITAMINS

By J. S. Foster and J. W. Murfin

From the Standards Department, Boots Pure Drug Co. Ltd., Airdrie Works, Airdrie, Scotland

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Quantitative separation of mixtures of organic bases into their components has been achieved by ion-exchange chromatography on alginic acid. This simplifies the routine control of nicotinamide and aneurine and pyridoxine hydrochlorides, when these are present in certain pharmaceutical preparations.

The successful application of alginic acid to the separation of organic bases from non-basic material (Foster and Murfin, 1961) led to an attempt to extend the work to pharmaceutical preparations containing some vitamins of the B group. It was hoped to achieve fractionation and quantitative separation of pyridoxine, nicotinamide and aneurine when present in admixture with other materials, and subsequently to assay each of these separated components spectrophotometrically. This approach promised a simpler and quicker method of estimation than the official microbiological assays.

Whilst the formaldehyde treatment of alginic acid had proved moderately successful, material thus prepared proved difficult to free from matter absorbing in the ultra-violet region of the spectrum. Prolonged use, moreover, tended to lead to disintegration into "fines". An alternative means of preparing alginic acid for ion-exchange was therefore sought.

Experimental

Preparation of Alginic Acid Ion-Exchanger

The following method, based on that of Specker and Hartkamp (1953) was found suitable.

Dust alginic acid (40 g.) slowly on to a solution of sodium hydroxide (12 g.) in water (450 ml.) with vigorous stirring. Continue stirring until a glutinous, homogenous solution results. Add this dropwise through a nozzle approximately 1·5 mm. internal diameter to 10 per cent w/w hydrochloric acid, stirring continuously during and for 15 min. after addition. Decant the liquid from the precipitate through a muslin filter over the mouth of the vessel and wash the precipitate until the washings are neutral to litmus and chloride-free. Wash the alginic acid three times with acetone, steep in the same solvent overnight and dry at a temperature not exceeding 50°.

Powder the product—hard, translucent pellets, light yellow-brown in colour—in a mill with a high-speed rotating blade and select a mesh fraction suitable for use.

Preparation of Columns

Use Pyrex tubes of 2 cm. internal diameter. Soak the prepared alginic acid (4 g. 72–85 mesh B.S. per column) in water until swelling is complete

(~ 4 hr.), then pack in suspension into the tubes previously plugged with cotton wool. Allow the alginic acid to settle and place second plugs on top. Wash the columns with 2N hydrochloric acid until the washings have no measurable extinction at the wavelengths subsequently to be employed and then with water until the effluents are neutral to litmus.

When not in use, leave the columns saturated with water.

General Procedure

Place the sample (10 ml. of aqueous solution) on the column, at 1 ml./min. and wash in with small volumes of water at the same flow-rate. Wash the column through with water (200 ml.) at the full flow-rate of up

TABLE I

RECOVERIES OF ANEURINE, NICOTINAMIDE AND PYRIDOXINE

	Wt. of compound taken (mg.)	Eluant (HCl)		Wave-length (mμ) extinction measured	Per cent recovery		
		Normality	Quantity (ml.)		1	2	3
Nicotinamide	15	0·005	500*	261	99·7	99·4	100·0
Aneurine	2	2·0	150	246	100·0	99·8	100·0
Aneurine, after passing 500 ml. of 0·005N HCl	2	2·0	150	246	100·0	99·8	99·8
Separation of Aneurine and Nicotinamide:							
1. Nicotinamide	15	0·005	500*	261	100·0	100·0	100·0
Aneurine	2	2·0	150	246	100·2	100·4	100·2
2. Nicotinamide	15	0·005	500*	261	100·0	100·0	100·0
Aneurine	2	2·0	150	246	100·0	99·8	100·0
Pyridoxine	5	0·005	500	291	100·0	100·0	100·0

* Dilute to 1 litre with eluant for measurement

to 20 ml./min. and then elute each base with hydrochloric acid of suitable strength at 12 ml./min. Completeness of elution and cleanness of separation are verified by spectroscopic measurements on small extra volumes of eluate.

Calculate the recovery of each base from the extinction of the eluate using the parent solution as a standard. Make all measurements against the eluting acid using 1 cm. cells except where otherwise stated.

Separation of Aneurine, Nicotinamide and Pyridoxine

Recovery experiments were made on the vitamins singly and in admixture. In addition, the separations were followed by the examination of 20 ml. fractions. The details and results are given in Table I and Figs. 1 and 2.

The weights per fraction of nicotinamide and pyridoxine hydrochloride were calculated from a two-point procedure.

	246 mμ	261 mμ	291 mμ
Aneurine hydrochloride E (1 per cent, 1 cm.)	416	—	—
Nicotinamide E (1 per cent, 1 cm.)	—	411	2·24
Pyridoxine hydrochloride E (1 per cent, 1 cm.)	—	48·2	427

Although the ultra-violet absorption spectrum of pyridoxine is pH sensitive over part of the range (Stiller, Keresztesy and Stevens, 1939),

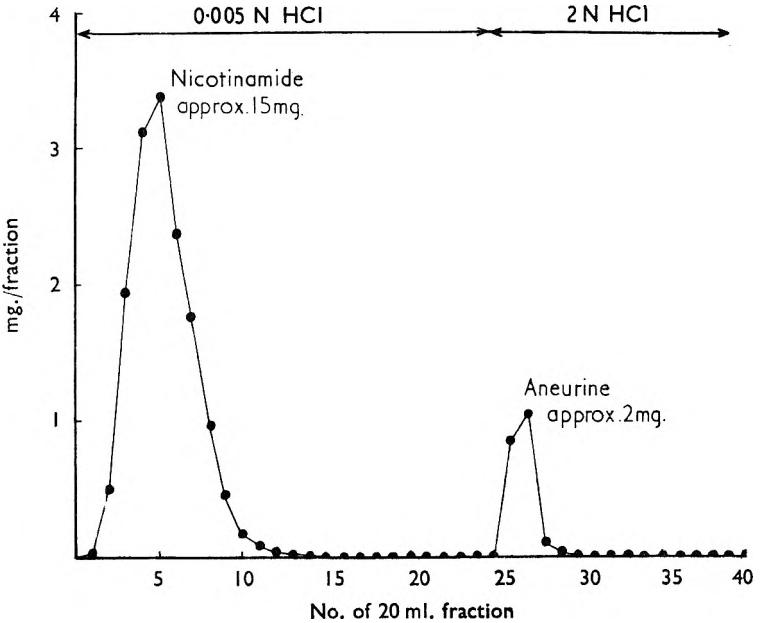

FIG. 1. Characteristic curves for the separation of nicotinamide (15 mg.) from aneurine HCl (2 mg.) Complete elution of nicotinamide, fractions 1–18; of aneurine, fractions 26–34. Elution of the first 25 fractions with 0·005 N HCl; the remainder with 2 N HCl.

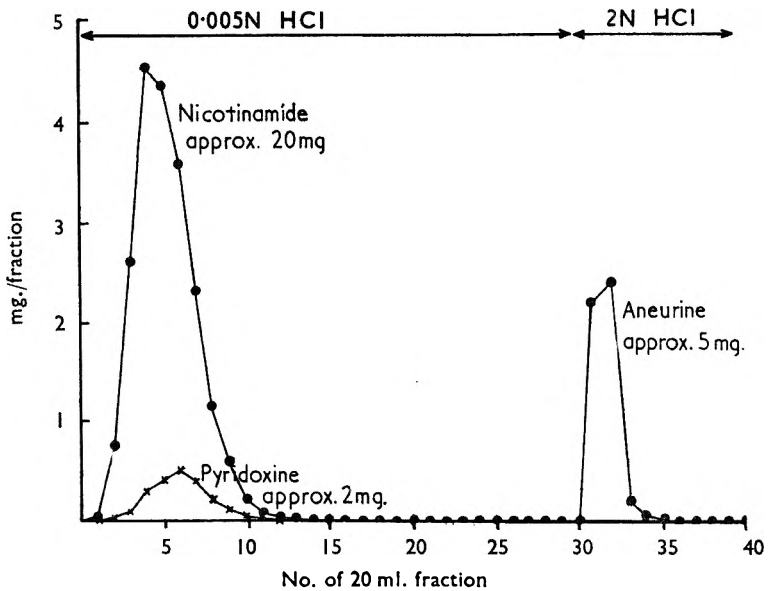

FIG. 2. Separation of nicotinamide (20 mg.) and pyridoxine HCl (2 mg.) from aneurine HCl (5 mg.) Loading in 10 ml. of 0·5 per cent w/w acetic acid. Complete elution of nicctinamide, fractions 1–17; of pyridoxine, fractions 1–14; of aneurine, fractions 31–36. Elution of the first 30 fractions with 0·005 N HCl; the remainder with 2 N HCl.

the extinctions at 261 and 291 mμ are constant in 0·004 to 0·006N hydrochloric acid.

Curves for nicotinamide and pyridoxine hydrochloride covering the wavelengths used are shown in Fig. 3.

Application to the Examination of Pharmaceutical Preparations

The procedures described are based on the assumption that no significant deterioration of aneurine has occurred, as may happen on long or unsuitable storage.

Tablets of Aneurine, Compound, B.P.C.

Take a sample of tablets and determine the average weight. Powder the sample and weigh accurately the equivalent of about seven

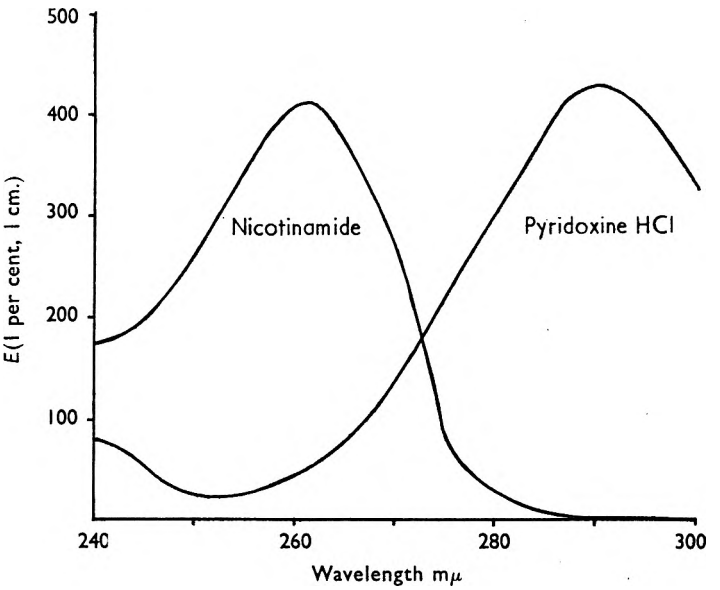

FIG. 3. Ultra-violet spectra of nicotinamide and pyridoxine HCl in 0·005 N HCl.

tablets. Extract the bases by agitating the powder continuously with 50·0 ml. of 0·5 per cent v/v acetic acid for 30 min. Filter (pH should lie between 3 and 4) and reject the first 10 ml. filtrate. Separate and estimate the nicotinamide and aneurine as detailed under general procedure and in Table I.

This procedure was carried out on a standard mixture of the tablet ingredients in which starch was used as the inert diluent, and then on three production batches of tablets. The results (Tables II and III) were compared with those obtained by the B.P.C. methods.

Tablets of Aneurine, Compound, Strong, B.P.C.

Slight modification of the assay above allows pyridoxine hydrochloride to be estimated in addition to the other two components. Use

100 ml. of 0·5 per cent v/v acetic acid for extracting the bases. Separate nicotinamide + pyridoxine from aneurine hydrochloride as detailed in general procedure, eluting the nicotinamide and pyridoxine with 500 ml. of 0·005N hydrochloric acid. Measure the extinction of this solution at

TABLE II

RECOVERIES FROM MIXTURES OF INGREDIENTS OF PHARMACEUTICALS

	Per cent recovery		
	1	2	3
Tab. Aneurine Co., B.P.C.			
Aneurine HCl	100·3	100·0	100·0
Nicotinamide	100·3	100·3	100·3
Tab. Aneurine Co. Strong, B.P.C.			
1. Aneurine HCl	99·7	99·1	—
Nicotinamide	99·9	99·9	—
2. Aneurine HCl	100·0	99·2	100·3
Nicotinamide	99·9	99·9	100·0
Pyridoxine HCl	99·0	99·0	99·0
Capsules of Vitamins, B.P.C.			
Aneurine HCl	99·7	100·0	100·4
Nicotinamide	100·3	100·2	100·2

291 mμ in a 4 cm. cell and the extinction of a twofold dilution at 261 mμ in a 1 cm. cell. Elute the aneurine with 250 ml. of 2N hydrochloric acid.

This modification was tried on a solution of aneurine, nicotinamide and pyridoxine and on two batches of tablets. An earlier method for aneurine and nicotinamide only, used 1 cm. cells throughout. This

TABLE III

ESTIMATION OF ANEURINE AND NICOTINAMIDE IN PRODUCTION SAMPLES

Product	Batch No.	Nicotinamide mg. per capsule or tablet				Aneurine hydrochloride mg. per capsule or tablet			
		Proposed method			B.P.C. method	Proposed method			B.P.C. method
		1	2	3		1	2	3	
Tabs. Aneurine Co., B.P.C.	1	14·8	14·8	14·8	14·7	0·93	0·93	0·93	0·89
		14·5	14·5	—		0·92	0·92	—	0·88
	2	14·9	14·9	—	14·8	1·03	1·03	—	1·01
	3	13·6	13·6	—	13·6	1·04	1·04	—	1·06
Tab. Aneurine Co, Strong, B.P.C.	1	18·9	18·9	—	19·0	5·05	5·05	—	5·3
	2	19·8	19·8	—	19·9	5·34	5·33	—	5·4
Capsules of Vitamins, B.P.C.	1	7·09	7·11	—	7·6	0·92	0·92	—	1·15
	2	7·07	7·07	—	7·3	1·01	1·01	—	1·14
	3	7·17	7·18	—	7·3	1·08	1·08	—	1·16
	4	7·05	7·06	—	7·6	1·00	1·00	—	1·20
	5	7·15	7·18	—	7·3	1·05	1·05	—	1·23
	6	7·07	7·07	—	7·5	1·09	1·09	—	1·07

method had been applied to a mixture of tablet ingredients, and to the same two batches of tablets.

The results are given in Tables II, III, IV.

Capsules of Vitamins, B.P.C.

Accurately weigh an amount of capsule contents equivalent to about 20 capsules. Add cyclohexane (20 ml.) and 0·15 per cent v/v acetic

acid (20 ml.). Warm and stir on a steam bath for 5 min. Transfer to a separator; wash the beaker out into the separator with alternate small volumes of cyclohexane and warm dilute acetic acid. Cyclohexane up to 60 ml. and dilute acetic acid up to 50 ml. may be used. Shake well and allow to separate. Filter the aqueous layer through a sintered-glass filter (No. 3 or 4 porosity) to remove undissolved riboflavine. Transfer the filtrate to a 100 ml. calibrated flask. Extract the cyclohexane layer with dilute acetic acid (3 × 15 ml.) using each extract to wash the filter and flask and adding each to the calibrated flask. Cool the flask and make up to volume with water. The pH of this solution should be 3–4. Place 10 ml. on an alginic acid column and carry out the separation and measurements for nicotinamide and aneurine by the general procedure using the details given in Table I.

TABLE IV

ESTIMATION OF ANEURINE, NICOTINAMIDE AND PYRIDOXINE IN PRODUCTION SAMPLES OF TABLETS OF ANEURINE CO. STRONG, B.P.C.

	Nicotinamide mg. per tablet				Aneurine hydrochloride mg. per tablet				Pyridoxine hydrochloride mg. per tablet		
	Proposed method			B.P.C. method	Proposed method			B.P.C. method	Proposed method		
B. No.	1	2	3		1	2	3		1	2	3
1	18·9	18·9	18·9	19·0	5·08	5·08	5·07	5·3	1·74	1·74	1·74
2	19·6	19·7	—	19·9	5·29	5·30	—	5·4	1·90	1·90	—

This procedure was applied to a standard mixture of constituents equivalent to the contents of about 20 capsules, then to six samples of production capsules. In the standard mixture Halibut Liver Oil, B.P., was used to supply the vitamin A requirement.

Attempts to modify the method so as to apply it to disintegrated whole capsules have not been successful.

The results obtained are shown in Tables II and III.

DISCUSSION

The procedure for capsules of vitamins is based on the assumption, inherent in the B.P.C. method for ascorbic acid, that there is negligible absorption of water-soluble vitamins into the shell of the capsule. Although our method leads to results for aneurine hydrochloride and nicotinamide lower than by the B.P.C. assays, the extraction procedure is efficient and does not destroy aneurine. In an earlier series of experiments, four standard mixes of capsule contents were examined, using formaldehyde-treated alginic acid. Recoveries of aneurine between 98·1 and 100·6 per cent and of nicotinamide between 99·7 and 100·5 per cent were obtained.

It seems likely that the absence of cross-linking in the exchanger ensures rapid and quantitative sorption and recovery of large organic molecules, whereas with synthetic cross-linked ion-exchangers quantitative recovery is often more difficult. This behaviour renders alginic

acid suitable for the resolution of a mixture of organic bases having different ionic charges.

Although the authors discarded formaldehyde-treated alginic acid, it should be mentioned that as an ion-exchanger it is not inferior to the precipitated material. Many of the experiments described above were made originally, on formaldehyde-treated alginic acid, with similar results.

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

The alginic acid used was the normal commercial grade. The column was capable of an indefinite number of operations, but occasional repacking might be necessary. The mesh size was not critical. A much wider range, 50–120 mesh, could be used but uniformity was necessary. The material might better be prepared by damping with aqueous alcohol of suitable strength and then granulating to the required mesh size.

Short Communication

ON THE STANDARDISATION OF THYROID B.P.

By C. A. Johnson and K. L. Smith

From the Standards Department, Boots Pure Drug Co. Ltd., Nottingham

Received May 23, 1961

The B.P. method for the standardisation of thyroid in terms of thyroxine-iodine is based on the work of Harington and Randall (1929) who claimed that the iodine in combination as thyroxine is related to the biological activity. When applied to the standardisation of tablets the method has given high and variable results; this has been ascribed to the presence of lactose (Doery, 1945) and the B.P. 1958 introduced an acid washing procedure to eliminate the possible interference of this substance when used as excipient. Subsequently this step in the analysis was introduced for official thyroid powder, since most of the commercial products are obtained in too concentrated a form and are suitably diluted with lactose.

In our hands this modification either for commercial thyroid powder or tablets has not given satisfactory results. This was reported to the appropriate committee of the Pharmacopoeia Commission and a critical examination of the procedure was started in an attempt to solve the problem.

Since a large number of determinations was likely, a less laborious method than the sodium carbonate fusion technique for converting organic iodine to iodide was sought. Such a method was found by modifying the flask combustion technique described by Johnson and Vickers (1959) for the determination of iodine in organic compounds. This gives results which are comparable with, but slightly higher than the classical method. Combustion and titration of the liberated iodide by the proposed method can be carried out in 20–30 min., which represents a considerable saving in time over the official method. The detailed procedure for the general analysis is given in the paper referred to above; this has been adapted for use in the presence of a large amount of organic matter by using a 2 litre flask and a sample weight of up to 0·8 g.

The original B.P. 1958 method, with this modification in combustion, was then applied to samples of thyroid before and after the addition of lactose and it was confirmed that the presence of lactose during hydrolysis markedly increases the apparent thyroxine content. We have also shown that a hydrolysis product of lactose itself precipitates under the conditions of the assay and occludes inorganic iodide thus producing a significant effect in the assay of thyroid for "thyroxine-iodine" content. When the acid washing stage of the B.P. Addendum 1960 was then included in some further determinations, it was found that even with thyroid which contained no lactose, the acid wash itself could cause a considerable lowering of "thyroxine-iodine" content. The results obtained are given in Table I although no particular significance can be attached to the magnitude of the loss, since this may vary from occasion to occasion and from operator

to operator. That losses do occur has been substantiated with a second sample of undiluted thyroid.

Hence it is apparent that the determination of "thyroxine-iodine" is not a satisfactory procedure to apply to lactose diluted thyroid and that the modification introduced by the B.P. to improve the assay has not been

TABLE I

THE EFFECT OF ACID-WASHING ON THE DETERMINATION OF "THYROXINE-IODINE" IN UNDILUTED THYROID POWDER

Per cent "thyroxine-iodine"	
Without acid-washing	With acid-washing
0·24	0·21
0·24	0·18
0·24	0·18
	0·21

successful. For standardisation of thyroid the U.S.P. relies upon a determination of total "iodine in thyroid combination". Such a determination would be unaffected by the nature and amount of diluent present. To assess the validity of this, and to compare the results obtained with those from "thyroxine-iodine" determinations, three samples of undiluted thyroid powder from the glands of oxen, pig and sheep respectively were assayed by both chemical and biological methods.

Several biological systems have been described for the assay of thyroidal substances but from our experience the choice of method lies between that

TABLE II

COMPARISON OF CHEMICAL AND BIOLOGICAL STANDARDISATION OF POWDERED THYROID

Sample	Per cent of "thyroxine-iodine"		
	Chemical	Biological (P = 0·95 limits of error)	
	Without acid washing	Rat goitre method	Total iodine
16354	0·11	0·13 (0·09–0·19)	0·22
Oxen	0·13	0·94 (0·75–1·11)	0·36
Pig	0·24	1·16 (0·93–1·46)	0·64
Sheep	0·21	1·23 (1·0–1·5)	0·49

based on the reduction in the asphyxiation time in mice (Smith, Emmens and Parkes, 1947) or that depending on the anti-thiouracil goitre effect in rats (Reineke, Turner, Kohler, Hoover and Beezley, 1945). Although of these the mouse anoxia method has the apparent advantage of being less laborious, less time consuming and calling for less skill, the results from it were more variable in our hands and we prefer the goitre prevention method.

Although it is desirable that a reference standard should simulate as nearly as possible the samples under examination we chose to make our comparisons against thyroxine sodium and thus avoid the difficulty of using a sample of thyroid as an arbitrary standard. In our assays both standard and samples were administered orally.

The biological and chemical results which have been obtained in the examination of ox, pig and sheep thyroid, together with those obtained on a commercial sample of unknown source, are shown in Table II. They illustrate the lack of relation not only between the biological assay figures and thyroxine iodine determined chemically but also between the biological activity and the total iodine.

The three samples of thyroid from oxen, pig and sheep were kindly supplied by Burroughs Wellcome and Co. The results of their own examination of these samples form the basis of a separate publication (Webb, 1961).

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COMPARISON OF BIOLOGICAL AND CHEMICAL ASSAY OF THYROID

By F. W. Webb

From the Wellcome Biological Control Laboratories, Dartford, Kent

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The biological and chemical examination of thyroid samples has demonstrated the inadequacy of the B.P. assay, which is based upon the determination of so-called "thyroxine iodine". It is suggested that, in the absence of specific methods for determining thyroxine and tri-iodothyronine in thyroid, the chemical assay should be replaced by a biological assay.

The mouse anoxia method is shown to be suitable for this purpose. The potencies of samples assayed thus over the past 3 years, together with chemical data and clinical comments are presented. The relative activities of the thyroid constituents 3,5,3′,5′-tetra-iodo-L-thyronine (L-thyroxine), 3,5,3′-tri-iodo-L-thyronine, 3,5-di-iodo-L-tyrosine, 3-mono-iodo-L-tyrosine, as determined by the mouse anoxia method, are in good agreement with their reported clinical effectiveness.

During 1958 it was found in these laboratories that the chemical assay for thyroid (B.P. 1958) gave results not always in agreement with the physiological activity in man. Most of the discrepancies were attributed to lactose used as a diluent which interfered with the assay, thus confirming the work of Doery (1945).

As a result, the chemical assay for thyroid was amended (B.P. 1958 Addendum 1960) to conform with that for thyroid tablets, in which the lactose is first removed by washing.

Some discrepancies, however, still remained, and were ascribed to differences in the relative proportions of biologically active constituents such as thyroxine and tri-iodothyronine. The greater activity of the latter is not taken into account in the B.P. assay.

This led us to examine several biological methods of assay for one suited to the routine standardisation of commercial samples and one which would give a better indication of clinical activity.

Since there may be species differences between thyroid derived from the three main commercial sources, ox, pig and sheep, which could lead to difficulties in their chemical or biological assay, samples from all three sources were examined.

Experimental Methods

Chemical

Determination of "Thyroxine Iodine" by the method described in B.P. 1958 and Addendum 1960.

Determination of Total Iodine, as for "thyroxine iodine", but ignoring the acid precipitation (B.P. 1958).

Biological

Oxygen Consumption Method in rats. Gaddum (1930).

Goitre Prevention Method in rats. Dempsey and Astwood (1943). Male albino rats, 100–140 g. in weight were used: the animals were killed by asphyxiation with carbon dioxide.

Mouse Anoxia Method. The method of Smith, Emmens and Parkes (1947) was modified. Male albino mice in a 2 g. weight range, within the limits of 15–20 g. were divided into 6 equal groups, each of 16–20 animals. A 3 + 3 assay design was used. Occasionally a further similar

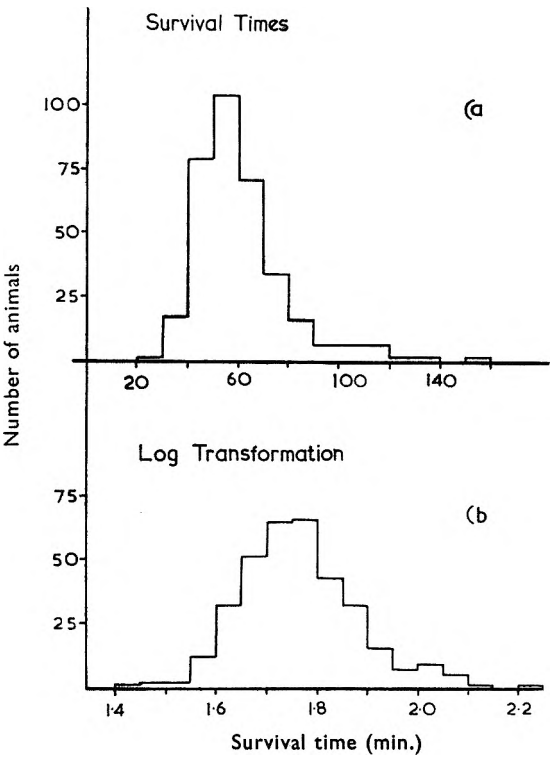

FIG 1. a. The distribution of survival times of 343 animals which received the same dose of thyroid.
b. Distribution of a log transformation of the data used in Figure 1a.

group was kept as a control. Using a dose ratio of 2:1, doses of the standard and test preparations were given by subcutaneous injection on three alternate days, each animal in a group receiving the same dose in 0·5 ml. solvent irrespective of weight. Powdered thyroid was administered in suspension, and thyroglobulin in solution, in distilled water. The doses used were equivalent in effect to 20, 10 and 5 μg. of sodium-L-thyroxine per mouse per injection.

L-Thyroxine sodium salt (anhydrous), 3,5,3′-tri-iodo-L-thyronine sodium salt, 3,5-di-iodo-L-tyrosine and 3-monoiodo-L-tyrosine were dissolved in 0·1N sodium hydroxide solution and diluted to give a final alkali concentration of approximately 0·001N.

The experiment was conducted at 23° on the second day after the last injection. The mice were put into separate blood transfusion bottles (volume 535 ± 4 ml.) which were sealed with rubber stoppers. Each bottle contained 50 ml. of dry sawdust. The time of survival to the nearest half minute was recorded for each mouse, commencing at the closure of the bottle and terminating at the last visible respiration, which was usually preceded by marked convulsions. At the end of the experiment the mice were weighed to the nearest 0·5 g.

TABLE I

CHEMICAL AND BIOLOGICAL ASSAYS AND CLINICAL ASSESSMENTS OF COMMERCIAL SAMPLES OF THYROID

Sample No.	Total iodine	"Thyroxine iodine"		Biological determinations			
	As a percentage by weight of the stated amount of thyroid present			Potency expressed as per cent of thyroid tablets No. 13	Limits of error (P = 0·95)	Method	Clinical effect
		Washed	Unwashed				
Thyroid tablets							
1				37		Mouse anoxia	—
2				41		"	—
3				72		"	—
4				37		"	—
				34		Rat goitre	
				43		Rat O_2 consumption	
5		0·050		39		Mouse anoxia	—
6				44		"	—
7				109		"	+
8				116	86–155	"	
9		0·104		119	89–159	"	+
10		0·102		128	95–171	"	—
11		0·094		113	84–151	"	+
12		0·074		73	48–102	"	
13 (Standard)	0·328	0·102		100	—		
Powdered Thyroid							
14		0·055	0·140	19	14–26	"	—
15		0·075	0·120	30	15–46	"	
16		0·069	0·097	64	46–94	"	
17		0·081	0·095	89	63–123	"	
18	0·260	0·069	0·101	85	61–113	"	
19	0·265	0·092		102	74–142	"	

— = Ineffective. + = Satisfactory

Before the actual assay a preliminary test to determine the approximate activity of the material was usually performed on 6 groups of animals with 3 to 5 mice per group, at dose levels equivalent to 40, 10 and 2·5 μg. of sodium-L-thyroxine.

The standard used for all assays was a production batch of ox thyroid tablets, 2 gr. (Sample 13, Table I).

RESULTS

Mouse Anoxia Method: Distribution of Survival Times

Figs. 1a and 1b show the distribution of survival times and log survival times respectively of a group of 343 mice which had received the same dose of thyroid on 3 alternate days in a series of mouse anoxia tests.

Taking the χ^2 class intervals, mean $\pm \frac{1}{4}$, $\frac{1}{2}$, 1 and 1·5 times the standard deviation, the distribution of survival times in min. is significantly skew ($P < 0{\cdot}001$, $\chi^2 = 55{\cdot}6$ with 9 degrees of freedom). The distribution of the log survival times is not significantly skew ($0{\cdot}1 > P > 0{\cdot}05$ and $\chi^2 = 15{\cdot}10$ with 9 degrees of freedom) and this is in agreement with the findings of Basil, Somers and Woollett (1950). All the mouse anoxia experiments have therefore been calculated on the basis of log survival time. Although the weights of the mice were initially within a 2 g. weight range, at the end of the test the range had widened such that it was found necessary to correct all responses for body weight by covariance analysis.

TABLE II

CHEMICAL AND BIOLOGICAL ASSAYS OF THYROID SAMPLES FROM OX, PIG AND SHEEP

	Thyroid sample		
	Ox	Pig	Sheep
Chemical Assays (Values as percentage by weight)			
Total iodine	0·345	0·581	0·475
"Thyroxine iodine"	0·107	0·173	0·155
Thyroxine*	0·130	0·182	0·118
Tri-iodothyronine*	0·034	0·076	0·034
Biological Assays			
Mouse anoxia method (Potency in terms of "ox" sample)	100	255	113
Limits of error ($P = 0{\cdot}95$) as percentage of "ox" sample	—	162–399	77–167

* Results obtained by Mr. Devlin, Canadian Department of National Health and Welfare and published by permission of Dr. N. R. Stephenson.

Comparison of Biological, Chemical and Clinical Data on Thyroid Samples from Various Sources

Table I shows the results obtained in the chemical and biological assay of 13 samples of thyroid tablets, and 6 samples of powdered thyroid obtained from various sources. The table also includes clinical comments where these are known.

A marked variation occurs in the biological activity of the samples, and this is reflected in their clinical effects. There is also variation in the results of the chemical assay: such figures moreover are not always related to biological activity.

Studies on Thyroid from Ox, Pig and Sheep

Table II shows the results of biological and chemical assays obtained on samples of thyroid from ox, pig and sheep.

The method used by Mr. Devlin for the estimations of thyroxine and tri-iodothyronine is based upon the enzymatic hydrolysis of the thyroid followed by chromatographic separation, and spectrophotometric determination utilising the reaction with ceric sulphate-arsenious acid reagent.

In the chemical assays the results obtained for the sample of pig thyroid are higher than those for the other two samples. Further, the biological potency of the sample of pig thyroid is more than twice that of the other two materials.

The Relation Between Chemical and Biological Assays

Table III shows ratios of biological potency to "thyroxine iodine" (B.P. method) and to total iodine (U.S.P. method) calculated on the basis of taking that of the standard as unity. A significant deviation from unity

TABLE III

RELATIONSHIP BETWEEN CHEMICAL AND BIOLOGICAL ASSAYS

Sample No.	Biological potency / "Thyroxine iodine" (B.P.)	Biological potency / Total iodine (U.S.P.)
	Limits of error (P = 0·95)	
9	1·16 (0·87–1·56)	
10	1·28 (0·95–1·71)	
11	1·23 (0·92–1·64)	
12	1·01 (0·66–1·41)	
13 (Standard)	1·00 –	1·00
14	0·36 (0·27–0·48)	
15	0·41 (0·20–0·63)	
16	0·95 (0·68–1·39)	
17	1·11 (0·79–1·55)	
18	1·26 (0·90–1·67)	1·07 (0·76–1·42)
19	1·14 (0·83–1·58)	1·27 (0·92–1·75)
Ox	used as "standard" 1·00	1·00
Pig	1·58 (1·004–2·47)	1·51 (0·96–2·37)
Sheep	0·78 (0·53–1·15)	0·82 (0·56–1·21)

(samples 14, 15 and pig thyroid) indicates that the chemical method of assay (B.P.) gives results which are not in agreement with biological potencies determined by the mouse anoxia method. In no other instance was there a significant difference.

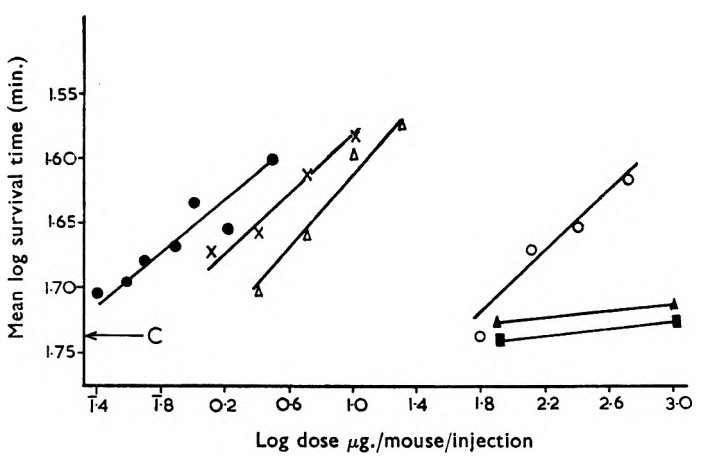

FIG. 2. Dose response curves of thyroid and thyroid constituents using the mouse anoxia method.

△ L-Thyroxine sodium salt. ● 3,5,3′-Tri-iodo-L-thyronine. (Na salt). ■ 3,5-Di-iodo-L-tyrosine. ▲ 3-Mono-iodo-L-tyrosine. X Thyroid tablets sample No. 13 (Standard). ○ Thyroglobulin. C Control (untreated mice).

The responses for thyroxine tablets sample No. 13 are plotted on the basis of sodium thyroxine content calculated from B.P. 'thyroxine iodine' values.

Relative Potencies of Thyroid and Thyroid Constituents

Using the mouse anoxia method the relative activities of several thyroid constituents were obtained. Fig. 2 shows the mean log survival times of groups of 10–20 mice plotted against log dose in μg./mouse for each substance. The dose response curves of active materials show no significant deviation from parallelism. 3-Mono-iodo-L-tyrosine and 3,5-di-iodo-L-tyrosine are inactive. 3,5,3′-Tri-iodo-L-thyronine sodium salt is 4·5 times as potent as L-thyroxine sodium salt (w/w).

Data obtained for sample No. 13 are plotted in terms of the expected sodium thyroxine content, calculated from the "thyroxine iodine" (B.P. 1958). The activity of the sample is 206 per cent of that expected (limits of error, P = 0·95 from 135–318 per cent). This was confirmed using the rat goitre prevention method (potency 246 per cent, limits of error, P = 0·95, from 116–409 per cent).

The potency of the thyroglobulin sample in terms of L-thyroxine is 2 per cent.

Discussion

The basis for the standardisation of commercial thyroid preparations as described in the British and United States pharmacopoeias is a chemical assay and occasionally marked differences have been reported between the results of the chemical and biological assays (Gaddum and Hethering-ton, 1931).

The method of assay in the British Pharmacopoeia 1958, Addendum 1960, takes the acid insoluble iodine content (so called "thyroxine iodine") as the measure of activity, whilst that of the United States Pharmacopeia XVI uses the total iodine in organic combination.

The present method of assay, first described in the British Pharmacopoeia 1932, Addendum 1936, is based upon the work of Harington and Randall (1929). It involves the separation of clinically active, acid insoluble thyroxine from clinically inactive, acid soluble di-iodotyrosine. This takes no account of the very potent thyroid hormone, 3,5,3′-tri-iodo-L-thyronine, discovered by Gross and Pitt-Rivers (1952).

The method described in the U.S.P. XVI is based upon a relation, empirically determined, between the total iodine content of a sample and its physiological activity, as shown by its effect on myxoedema in humans by Means, Lerman and Salter (1933). It is assumed that in samples from different species there is the same proportion of thyroxine, tri-iodothyronine and inactive iodinated organic compounds such as 3-mono-iodo-L-tyrosine and 3,5-di-iodo-L-tyrosine. 3,3′-di-iodothyronine and 3,3′,5′-tri-iodothyronine also have been found in the thyroid (Roche, Michel, Wolf and Nunez, 1956) and are inactive (Gemmill, 1956, and Stasilli, Kroc and Meltzer, 1959). The results obtained in Dr. Stephenson's laboratory for the thyroxine and tri-iodothyronine contents of 3 samples of thyroid from ox, pig and sheep (Table II) do not indicate that this is so.

The results of the chemical assay of three thyroid powders (Table II), are not in good agreement with the biological potencies, and with the pig thyroid sample the difference is statistically significant.

In most instances in Table I the biological potencies relative to the working standard are of the same relative order as the "thyroxine iodine" values, although in two thyroid samples, numbers 14 and 15, the differences are significant, $P < 0.05$ (Table III). In the few instances where total iodine values are quoted they are in reasonable agreement with the biological potencies. It is possible therefore that in some thyroid samples the total organically bound iodine is related to biological potency. This cannot obtain where decomposition of active constituents or adulteration with iodinated casein has taken place; the ratio of organically bound iodine to physiological activity in iodinated casein is dependent upon the conditions under which the iodination is carried out.

Since the biological potency of the standard (sample No. 13) is 206–246 per cent of the potency expected from analysis of "thyroxine iodine" (B.P. 1958) it follows that for most of the samples examined "thyroxine iodine" does not provide a valid indication of potency in terms of L-thyroxine. This confirms the observations of Frieden and Winzler (1948).

Therefore, in our opinion, neither of the methods at present described in the British Pharmacopoeia or the United States Pharmacopeia for the assay of thyroid is valid, as both are capable of providing misleading indications of physiological activity. In theory, the quantitative estimation of the two important thyroid hormones, 3,5,3′-tri-iodo-L-thyronine and 3,5,3′,5′-tetra-iodo-L-thyronine (L-thyroxine) should provide a better chemical assay for thyroid.

The results in Fig. 2, obtained by the mouse anoxia method, show that the activities of several iodine-containing compounds found in the thyroid are in good agreement with their reported clinical effectiveness.

3,5,3′-Tri-iodo-L-thyronine, which was approximately 4·5 times as effective as L-thyroxine in mice, has a potency variously reported as 3–10 times more effective than thyroxine in man, in increasing oxygen consumption (Gross, Pitt-Rivers and Trotter, 1952, Asper, Selenkow and Plamondon, 1953) and 5–10 times more potent than L-thyroxine in man in its effect on plasma cholesterol levels (Boyd and Oliver, 1960). The clinical inactivity of 3,5-di-iodo-L-tyrosine (Strouse and Voegtlin, 1909) is also reflected in the mouse anoxia test.

The present studies show therefore that the mouse anoxia method gives results which are in good agreement with clinical activity. The assay is easy to perform, the apparatus required is cheap and simple, and results are obtained within 7 days. Over a period of 3 years they have been reasonably consistent and the statistical weight per mouse has remained at about 1·5. We believe that the mouse anoxia method is suitable for the routine standardisation of thyroid.

Acknowledgements. I would like to thank Dr. G. E. Foster for the chemical assays of thyroid, Dr. N. R. Stephenson for his permission to use data obtained in his laboratories, on the chemical determinations of thyroxine and tri-iodothyronine in the samples of ox, pig and sheep thyroid, and Mr. R. E. A. Drey for the preparation of the sample of thyroglobulin.

I am also grateful to Dr. G. A. Stewart for his encouragement and interest in these studies, and Miss E. Tate for statistical analyses. I wish to thank the staff of the Wellcome Biological Control Laboratories for technical assistance.

The samples of thyroid from ox, pig and sheep used in these studies were also examined by Boots Pure Drug Co. Ltd.

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The papers were presented by MR. K. L. SMITH and MR. WEBB. The following points were made in the discussion.

Not all strains of mice were suitable for the mouse anoxia method. The mice needed to be kept at constant temperature throughout the assay. When compared with a reference thyroid sample in mice, oral administration of pig thyroid had given a potency within 5 per cent of that obtained by the subcutaneous route. Lactose should be replaced as a diluent by calcium phosphate which does not interfere with the chemical assay. There are formidable manipulative difficulties, caused largely by the three filtrations, in the B.P. assay, and inconsistent results have been encountered although there had been rigid adherence to B.P. conditions. A method of determining the physiologically active constituents of thyroid, viz., thyroxine and 3,5,3-tri-iodo-thyronine by paper chromatography was being investigated. Two difficulties had to be overcome; 1, the thyroid protein must be hydrolysed quantitatively to amino-acid and 2, there must be a minimum of di-iodination in the assay.

THE STRENGTH OF COMPRESSED TABLETS

III. The Relation of Particle Size, Bonding and Capping in Tablets of Sodium Chloride, Aspirin and Hexamine

By E. Shotton and D. Ganderton

From the Department of Pharmaceutics, School of Pharmacy, University of London, 29–39 Brunswick Square, London, W.C.1

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Examination of the fracture of tablets in a strength test has revealed two types of failure. Where the interparticulate bond is strong, fracture occurs across the grains and the strength of tablet is a simple function of the particle size. If the interparticulate bond is weak, fracture occurs around the grain and particle size has little effect. A thin coating of stearic acid was used to weaken the interparticulate bond when the effect of grain size on strength disappeared or was reversed. The relation of bonding and capping is discussed and it is shown that capping can be reduced by weakening the interparticulate bond.

Powdered materials can be aggregated by pressure so that the individual particles bond together. The strength of the resultant tablet depends upon a number of factors, one of which is the initial particle size of the material. This factor has been investigated by Nelson (1957), who used an empirical cleavage test on the tablet, and by Forlano and Chavkin (1960), who used disintegration and tumbling tests to assess their results.

To account for the great discrepancy between the practical and theoretical strengths of materials, Griffith (1920) postulated that stress concentrations occurred at flaws in the solid which were proportional to a function of the flaw length. This concept was developed by Orewan (1949) who suggested that the strength (S_o) of a brittle, polycrystalline material is inversely proportional to the square root of the mean grain size (L).

$$S_o = KL^{-\frac{1}{2}}, \text{ where K is a constant} \quad .. \quad .. \quad (1)$$

It was assumed that (i) failure occurred at a flaw within the grain, (ii) that the grain boundary would resist the propagation of the failure to adjacent grains and that (iii) the stress required to overcome this resistance was inversely proportional to the square root of the grain size. With the introduction of the dislocation theory of the deformation of crystals (Taylor, 1934), this concept was modified by Petch (1953), who proposed the relation:

$$S_o = S_1 + KL^{-\frac{1}{2}} \quad .. \quad .. \quad .. \quad (2)$$

where S_1 is an expression of the applied stress required to move a dislocation along a glide plane. These concepts were successfully applied to various systems; to iron by Petch and to certain ceramic materials by Knudsen (1959).

This paper describes investigations of the possible application of these hypotheses to pharmaceutical tabletting systems and aspirin, sodium chloride and hexamine were chosen because of their differing physical properties.

TABLE I

SUMMARY OF THE COMPRESSIVE FORCES IN THE PREPARATION OF TABLETS FROM DIFFERENT SIZE FRACTIONS OF SODIUM CHLORIDE, ASPIRIN AND HEXAMINE

Mesh	Applied pressure (kg. cm.$^{-2}$) P_a	Transmitted pressure (kg. cm.$^{-2}$) P_b	Mean compaction pressure $P_m = \frac{P_a + P_b}{2}$ kg. cm.$^{-2}$	$\frac{P_b}{P_a}$	Ejection force Fe (kg.)	Porosity per cent	Applied force—transmitted force $F_a - F_b$ (kg.)	Weight of tablet W (g.)
Sodium chloride								
30–40	809	683	746	0·844	69	15·9	162	1·0952
40–60	840	703	772	0·837	74	16·0	176	1·0944
60–80	855	712	784	0·833	76	16·5	183	1·0894
–80	867	723	795	0·834	77	16·9	185	1·0856
Aspirin								
20–30	946	666	806	0·704	—	4·5	361	0·7071
30–40	939	656	798	0·699	—	4·6	363	0·7062
40–60	963	661	812	0·686	—	4·9	388	0·7066
60–80	981	663	822	0·676	—	5·2	408	0·7065
Hexamine								
20–30	810	622	689	0·768	98	4·8	241	0·6611
30–40	825	628	727	0·761	108	5·1	253	0·6611
40–60	838	625	732	0·746	113	5·1	273	0·6615
60–80	845	624	735	0·738	113	5·4	284	0·6592

All values are the mean of six results. For any material, the machine setting is constant.

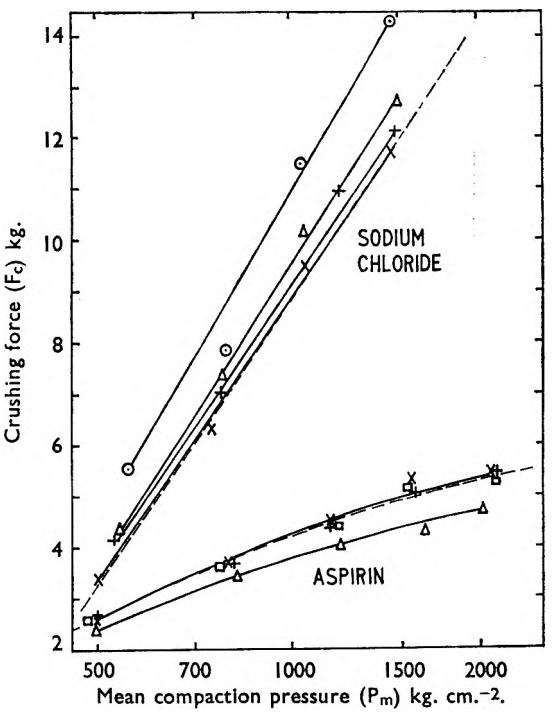

FIG. 1. Relation between mean compaction pressure (P_m) and crushing (F_c) for size fractions of sodium chloride and aspirin. Broken lines indicate results from a hopper-fed, mechanically driven series (30–40 mesh).

□ 20–30 mesh △ 60–80 mesh
× 30–40 mesh ○ –80 mesh
+ 40–60 mesh

Experimental and Results

Using British Standard sieves, sized fractions of sodium chloride, hexamine and coarse crystalline aspirin were separated and collected. The aspirin and sodium chloride were not further treated but the hexamine was dried at 55° and 2 cm. Hg absolute for 2 hr. before compression. This gave a strongly bonding, reproducible material.

The separate size fractions of each material were compressed on an instrumented tablet machine (Shotton and Ganderton, 1960a) over a pressure range of 500–2,000 kg. cm.$^{-2}$ in a $\frac{1}{2}$ in. diameter punch and die set. Mean values of applied pressure, transmitted pressure and ejection force were obtained for the compression of six tablets of each size fraction

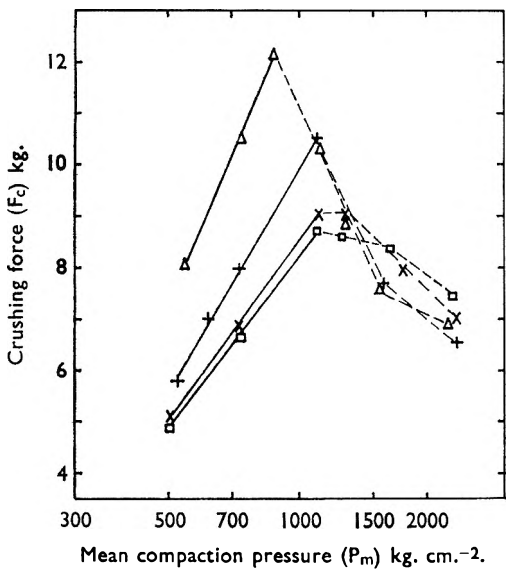

FIG. 2. Relation between mean compaction pressure (P_m) and crushing force (F_c) for size fractions of hexamine. The broken lines link results obtained from laminated compacts.

□ 20–30 mesh + 40–60 mesh
× 30–40 mesh △ 60–80 mesh

at each pressure level. The materials were fed to the die by hand and compressed by manually turning the machine. The weight of fill was calculated from the density to give a tablet of 0·4 cm. thickness at zero porosity. Each tablet was weighed, its dimensions measured, and finally the strength estimated using the crushing test previously described (Shotton and Ganderton, 1960a). A typical set of results is given in Table I and the relation of crushing strength and mean compaction pressure for the whole series is summarised in Figs. 1 and 2. The results of 30–40 mesh fractions of sodium chloride and aspirin compressed using hopper feed and mechanically driven conditions are superimposed as dotted lines on Fig. 1.

The mean particle size of each fraction was measured microscopically by taking the mean diameter of 800 particles. Some difficulty was encountered with hexamine because of the irregularity of shape and some aggregation. Fig. 3 gives the relation of particle size and strength.

In subsequent experiments, a dilute solution of stearic acid in light petroleum (100–120°) was progressively added to hexamine in a rotating

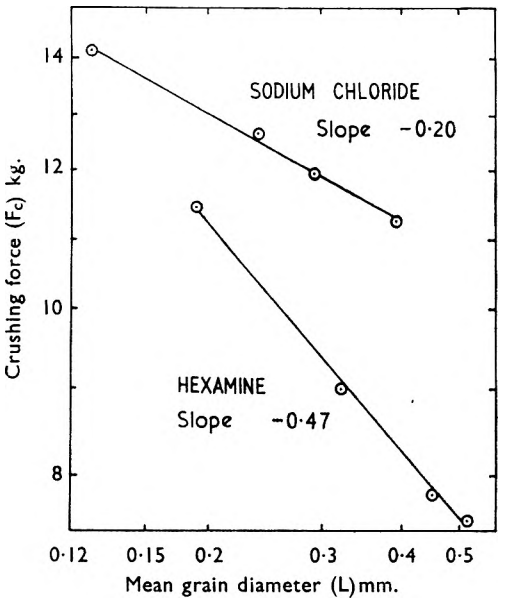

FIG. 3. The effect of grain size on the strength of tablets of sodium chloride and hexamine.

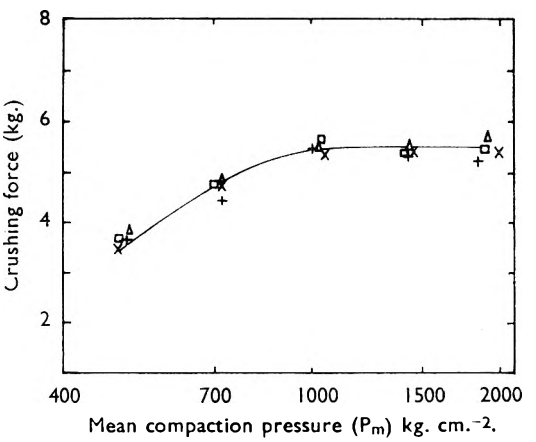

FIG. 4. The relation between the mean compaction pressure (P_m) and the crushing force for size fractions of hexamine coated with stearic acid.

□ 20–30 mesh + 40–60 mesh
× 30–40 mesh △ 60–80 mesh

coating pan until the dryweight percentage of stearic acid was 0·2. The particulate material was dried in an oven for 1 hr. at 60°, resieved and compressed using the same weight of fill as for the uncoated material. Sodium chloride was similarly coated and compressed. The results are presented in Figs. 4 and 5. Subsequently, a fraction of the hexamine was stripped of stearic acid using light petroleum, dried under vacuum as before, and recompressed. The results did not differ significantly from an untreated fraction of the same size range.

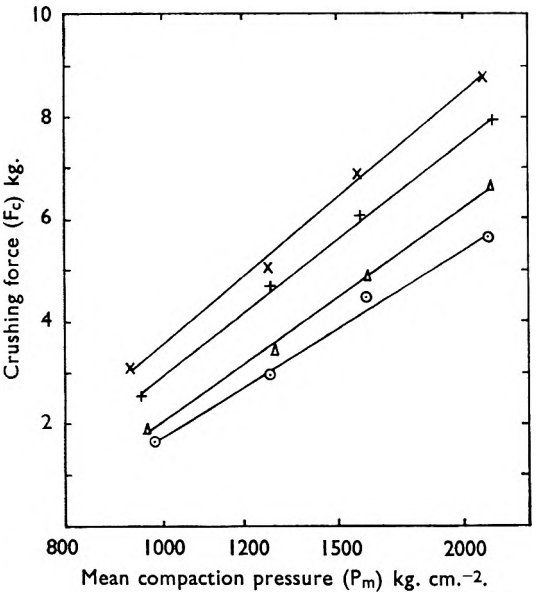

FIG. 5. The relation between the mean compaction pressure (P_m) and the crushing force (F_c) for size fractions of sodium chloride coated with stearic acid.

× 30–40 mesh △ 60–80 mesh
+ 40–60 mesh ○ −80 mesh

Hexamine, which in the first experimental series had shown marked capping and lamination, was now compressed in a die used for compressing discs under vacuum for spectrophotometric analysis. A 30–40 mesh size fraction was compressed under both normal and evacuated conditions using a hydraulic press. In the latter series a vacuum of 0·6 mm. Hg absolute was applied under standard conditions before compression and maintained during the application of the load. The dimensions of these compacts were measured and the crushing strength estimated. The results are presented in Fig. 6.

DISCUSSION

The increase in ejection force and force lost to the die wall (the difference between applied and transmitted forces), with decrease in particle size is shown in Table I. Since these forces derive from the shear of the area in proximity to the die wall during compression or ejection, this increase

must be due either to (*a*) an increase in total contact area with the die wall or (*b*) an increase in the effective shear strength. The former seems unlikely since, as Table I also shows, the voidage increases with decrease in particle size. However, the contact area will be composed of smaller areas intersected with pores and grain boundaries. The size of these individual areas will decrease with particle size and this will influence the shear strength in a manner indicated by Orewan's theory. The work of Train and Hersey (1960) and Long (1960) is a significant contribution to the theory of die-wall behaviour but insufficient is known about the actual

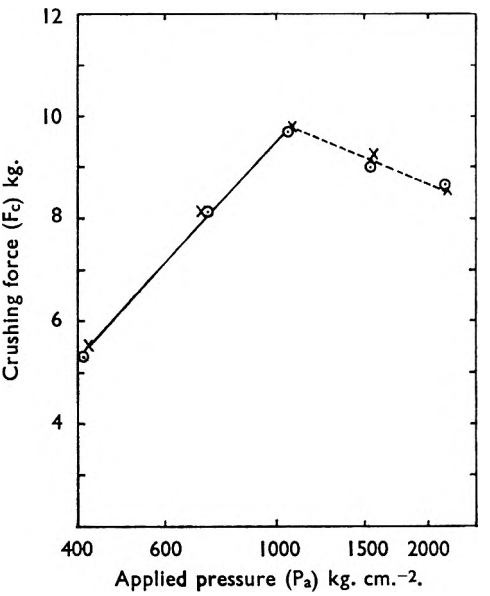

FIG. 6. The compression of hexamine under normal and evacuated conditions. The broken lines link results obtained from laminated compacts. (× normal, ○ evacuated.)

area in contact with the die wall and the change in the distribution of forces within the die with variation of particle size for the further elucidation of these effects.

The close agreement shown in Fig. 1 between the strength of tablets produced by a hand and a motor driven machine suggest that variation in the speed of compression inherent in the former method is not significant in the present work. In Figs. 1 and 2 the particle size of hexamine and sodium chloride affects the tablet strength in a manner indicated by Orewan's theory. With aspirin, however, the grain size has no effect except in the instance of the finest powder, where the strength of the tablet is lower. Examination of the tablet fracture under the microscope shows two types of behaviour. With sodium chloride and hexamine failure takes place to some extent across rather than round the grain, indicating a strong interparticulate bond. The extent of cross-grained failure increases as the applied pressure increases. A similar transition

has previously been shown (Shotton and Ganderton, 1960b) in a sucrose granulation. Aspirin tablets break round the grain indicating a weak interparticulate bond which yields before failure occurs across the crystal. Under these conditions, grain boundary resistance will not be effective and the strength will be independent of it. The lower strength of the aspirin tablets produced from the finest fraction may be due to a weakening effect of entrapped air. Long and Alderton (1960) have shown that, with some materials, this effect becomes more important as the particle size is reduced.

The relation of grain size and tablet strength is given in Fig. 3. For hexamine, the slope is $-0{\cdot}47$. This is in very close agreement with the equation (1) where S_0 is measured by the crushing force, F_c. For sodium chloride, the slope is $-0{\cdot}2$. However the data for both sodium chloride and hexamine satisfies the equation (2).

The difference in the behaviour of sodium chloride and hexamine on the one hand and aspirin on the other derives from the strength of the interparticulate bond and its effect on failure. Thus, grain size effects should entirely disappear if the interparticulate bond is sufficiently weakened. This weakening was produced in sodium chloride and hexamine systems using a thin coat of stearic acid, and Fig. 4 shows that, with a coated batch of hexamine, the strength of the tablet is independent of the grain size. The argument is further supported by an examination of the fractures which showed that failure occurred around rather than across the grain.

In contrast to coated hexamine, sodium chloride coated with stearic acid produced the strongest tablets from the coarsest fraction; a virtual inversion of the effect described above. However, the sodium chloride crystal is stronger than that of hexamine and is cubic, thus presenting relatively sharp edges. In compression, the load per particle/particle contact will increase with increase in particle size. Thus, in a coarser fraction there will be a greater tendency to penetrate the interparticulate film of stearic acid to form strong welds and so a stronger tablet.

The Capping of Hexamine

At a pressure of about 1100 kg. cm.$^{-2}$ hexamine tablets cap and laminate, an effect which then increases progressively with increase in pressure. Although the strength test on a laminated compact must be regarded with caution, the results in Fig. 2 indicate that the disruptive processes are more marked in the case of finer powders despite their higher intrinsic particulate strength. A practical outcome of the results is that, in the production of a hexamine tablet of acceptable strength, capping can be reduced and the effective strength maintained by reducing both the particle size and the applied pressure.

The results given in Fig. 6 for the compression of hexamine under normal and evacuated conditions show identical strength and capping characteristics. We can assume, therefore, that entrapped air is not responsible for capping in this instance. This assumption is further justified by the elimination of capping in the hexamine coated with stearic

acid. The geometry of the deforming system will be roughly the same and, if entrapped air was the causative agent, capping should still occur. Although the theories of capping proposed by Train (1956) and Long (1960) differ in fundamental respects, both agree that capping occurs through stresses produced by compression. Train considered that capping occurred on ejection because part of the tablet underwent radial recovery while the remainder was still confined by the die. Long considered that the stress pattern produced by radial forces after the withdrawal of the punches was the mechanism involved.

The variations in the behaviour of the materials studied may be explained by the following mechanisms. Sodium chloride, which produced strongly bonded compacts without capping, can elastically or plastically accommodate the strains produced by the recovery following compression and ejection. The minimum porosity attained in the series was 7 per cent and it is possible that these strains were relatively small. Hexamine, which also produced a strongly bonded compact, but with marked lamination, may be presumed to fail under recovery stresses and, because of the strength of the interparticulate bond, failure propagates across grains to produce extensive failure planes and laminae. The pressure required to consolidate the material completely was certainly exceeded and the relaxation was therefore large. Aspirin compressed to an equal degree, showed no tendency to cap. Because of the mechanical weakness of the interparticulate bond, the failure of an individual particle is not transmitted to neighbouring particles. Stresses are dissipated by a partial separation of the particles and extensive zones of failure are not produced. Thus, a weakening of the interparticulate bond, demonstrated by a round-grain fracture should reduce capping tendencies. This is clearly shown in Fig. 4 where capping in hexamine is eliminated by a thin coat of stearic acid. The introduction of stearic acid weakens the interparticulate bond to give a compacting system, similar to that of aspirin, in which failure occurs around the constituent particles; grain size effects and capping are absent and the resultant tablet is mechanically weak. Finally it is of interest to note that in a preliminary experiment, similar effects were found with a 30–40 mesh hexamine coated with 0·2 per cent hard paraffin.

Acknowledgements. The authors wish to thank Dr. David Train for his help in discussion and the D.S.I.R. for the award of a Research Studentship to Mr. D. Ganderton.

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

When lubrication with stearic acid was confined to the die wall the strength of the tablets obtained was the same as when no stearic acid was present. Tablets were tested within minutes of compression, and the interval was kept constant. There was slight attrition of the corners of the crystals, particularly cubic ones. Grain size was identified with particle size. Porosity of the tablets had been inferred from the density of the material, and the volume occupied by the tablets after compression

A NEW METHOD FOR THE DETERMINATION OF BARBITURATES

By I. M. Roushdi, H. Abdine and A. Ayad

From the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Alexandra University, U.A.R.

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The use of complexometric analysis for the determination of metal ions has suggested that this could usefully be applied to the assay of barbiturates. The method depends on precipitation of the barbiturate with excess of a metallic salt, followed by estimation of this excess complexometrically.

In earlier methods (Budde, 1934; Chavanne and Marie, 1953; Danielson, 1951; Kalinowski, 1935; Schulek and Rozsa, 1938; Stanier, Lapiere and De Tiege-Robinet, 1956) the barbiturates are precipitated as the silver salts, and excess silver nitrate determined by Volhard's method. In the modification of Mangouri and Milad (1947) precipitation is carried out in a sodium acetate buffer and the liberated acetic acid is neutralised with calcium carbonate before titration of the excess silver nitrate. When examined in our laboratory this method gave results which varied according to the amount of calcium carbonate used.

Another approach to the assay involves precipitation of the barbiturates as the mercuric salt with mercuric perchlorate. Excess reagent is again determined by Volhard's method (Pedley, 1950). Repetition showed that this method was reproducible only within 1 per cent when applied to barbitone and barbitone sodium: the end point moreover was found to be indistinct and easily over-stepped. Complexometric titration of the excess mercuric perchlorate was therefore investigated. After precipitating the barbiturate as described by Pedley the filtrate was treated with a known excess of standard magnesium complexonate

TABLE I

Application of the Hg and Zn methods to some barbiturates

Substance	Indicator	Results
Hg method—		
Barbitone	E[1]	99·4; 99·2; 98·8
	Cu P[2]	98·8; 99·3; 99·3
Barbitone sodium	E	99·5; 99·3; 99·3
	Cu P	98·1; 99·5; 98·9
Phenobarbitone	E	99·4; 99·1; 99·2
	Cu P	98·9; 99·6; 99·3
Phenobarbitone sodium	E	98·9; 98·5; 98·6
	Cu P	98·8; 98·6; 98·4
Zinc method—		
Barbitone sodium	E	99·4; 99·3; 98·7
Phenobarbitone sodium	E	99·2; 99·1; 98·7[3]
Amylobarbitone sodium	E	98·3; 99·0; 98·7
Pentobarbitone sodium	E	100·6; 100·4; 100·6

[1] Eriochrome black T.
[2] Cu [1-(2-Pyridylazo)-2-naphthol] complex.
[3] Result calculated on the assumption that the precipitate is 8 molecules phenobarbitone to 3 atoms Zn.

solution, buffered to pH 10, warmed to about 50° and titrated with standard EDTA using Eriochrome black T as indicator. Alternatively, the solution was buffered to pH 5 and the copper-PAN indicator used (Flaschka and Abdine, 1956abc). Reproducible results were obtained, and the end points were sharp. The results are shown in Table I.

Attempts were then made to use zinc as the precipitating ion instead of mercury. Zinc is much easier to titrate complexometrically as far as the pH of the titration, the interfering ions, and the available indicators are concerned (Schwarzenbach, 1955). Barbituric acid derivatives were quantitatively precipitated by zinc sulphate at pH 6, more acid solutions

TABLE II

RESULTS OF ASSAY OF A SAMPLE OF BARBITONE SODIUM BY DIFFERENT METHODS

B.P. method	Ag method	Hg method (*a*) Volhard	Hg method (*b*) complex (E.T.)	Hg method (*c*) complex (PAN)	Zn method
99·8	98·3	99·8	99·3	99·1	99·4
99·8	100·7	99·0	99·5	99·6	99·3
99·8	103·5	99·2	99·3	98·9	98·7
—	103·8	—	—	—	—
—	106·9	—	—	—	—
Mean 99·8	—	99·3	99·4	99·2	99·1

caused dissociation of the precipitate, while more alkaline solutions caused co-precipitation of basic zinc salts. The following procedure is recommended.

Weigh accurately about 0·4 g. of the sodium salt of the barbiturate, dissolve in water (about 100 ml.) and add boric acid buffer solution pH 6 (10 ml.), and heat nearly to boiling. Add 0·10M zinc solution (20 ml.) slowly with stirring, and keep at about 100° for 15 min. Cool and transfer to a 250 ml. volumetric flask, adjust to the mark and filter. Take 100 ml. of the filtrate, add buffer solution, pH 10 (10 ml.) and titrate with 0·02M EDTA solution using Eriochrome black T as indicator. Carry out a blank experiment omitting the barbiturate.

The results, shown in Table I, are calculated on the assumption that the complex is composed of one zinc atom and two molecules of the barbiturate. Table II compares the results of assay of a sample of barbitone sodium by the different methods.

Reagents

0·10M EDTA *disodium salt:* B.P. 1958. 0·10N *standard zinc solution:* Metallic zinc A.R. (6·538 g.) is dissolved by the aid of heat in the minimum amount of sulphuric acid A.R. The solution is cooled and made up to one litre with distilled water.

Buffer solutions. Borax buffer pH 6; Ammonium chloride-ammonium hydroxide buffer pH 10.

Indicators. Eriochrome black T (0·1 g.) is mixed with sodium chloride A.R. (20 g.). PAN (1-(2-pyridylazo)-2-naphthol) (0·1 per cent) in methanol.

Copper-EDTA : Mix equal volumes of 0·10M copper sulphate solution, and 0·10M EDTA.

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The paper was presented on behalf of the Authors by DR. HERSANT.

THE FLASK COMBUSTION TECHNIQUE IN PHARMACEUTICAL ANALYSIS: MERCURY-CONTAINING SUBSTANCES

By C. Vickers and J. V. Wilkinson

From the Analytical Development Group, Standards Department, Boots Pure Drug Co. Ltd., Station Street, Nottingham

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A method has been developed for the assay of mercury in organic samples using the flask combustion method followed by complexometric titration with a visual end point. The method has been applied to a wide range of mercury-containing materials used in pharmacy and agriculture.

The determination of mercury in organic compounds making use of decomposition by the flask combustion technique has received scant attention. Southworth, Hodecker and Fleischer (1958) successfully analysed some mercury compounds in this way finishing with a complexometric titration. Their attempts to use a visual end point were unsuccessful and they found it necessary to resort to an amperometric method. The method is not directly applicable to chlorine-containing compounds since these, on combustion, give mercurous chloride which must be oxidised before titration. If a visual end point method of titration could be developed, free from interference by halides the flask combustion assay should then be applicable to the many commercially important mercury-containing materials used in pharmacy and agriculture.

Experimental

Southworth, Hodecker and Fleischer (1958) found that the oxides of nitrogen produced when the combustion products dissolve in nitric acid, destroyed the indicators used in the complexometric titration. Attempts to eliminate this interference were unsuccessful. By analogy with the Rupp (1905) method of determining mercury, bromine water should prove a suitable absorbing liquid, even for those compounds giving rise to mercurous chloride on combustion, and this was found to be so. Attempts made to remove the excess bromine using either phenol or formic acid led to unsatisfactory end points when these solutions were titrated complexometrically. Removal of the bromine was finally achieved by simply drawing air through the solution. Sharper end points were thus obtained, with no increase in operator time over the formic acid method.

The choice of a suitable complexometric titration of the mercury was next considered. Although Southworth and his colleagues (1958) titrated with (ethylenedinitrilo) tetra-acetic acid solution, we preferred the more widely used EDTA. As an indicator solochrome black or 1-(2-pyridylazo)-2-naphthol (PAN) was satisfactory, the latter being preferred because the colour change was more easily seen at the end point.

In addition to simple titration with standard zinc solution a second method was considered. This involved back-titration with standard zinc solution, liberation of EDTA from the mercury complex by addition of potassium iodide and titration of the EDTA with standard zinc solution.

TABLE I

RESULTS OBTAINED ON PHENYLMERCURIC ACETATE (M.A.R. STANDARD)

	No. of determinations	Mean result	Range	Results by alternative procedure*
Sample No. 1—				
Platinum	8	59·3	59·1–59·4	59·4
Silica	2	59·3	59·2–59·4	—
Sample No. 2—				
Platinum	8	59·4	59·2–59·8	59·5
Silica	2	59·4	59·3–59·4	59·5

* The material is decomposed by refluxing with 4M sulphuric acid for 1 hr. Dilute potassium permanganate solution is added until the solution is just pink. After cooling hydrogen peroxide is added to remove the excess permanganate and the solution titrated with 0·1N ammonium thiocyanate solution using ferric ammonium sulphate solution as indicator.
This is the recommended method of the Microchemistry Group of the Society for Analytical Chemistry (1961).

TABLE II

RESULTS OBTAINED ON VARIOUS COMMERCIAL ORGANO-MERCURY COMPOUNDS BY THE FLASK AND OTHER METHODS

Substance	Mercury content per cent				
	Calculated from formula	By flask method			By alternative method
		No. of detns.	Mean result	Range	
Phenylmercuric nitrate B.P. ..	63·2	4	62·9	62·9–63·0	63·6[2]; 63·5[3] 63·0; 62·8[1]
Mersalyl sodium B.P.C.	39·7	4	39·2	39·1–39·3	39·4[1]
Mercurochrome B.P.C. 1954 (1)	26·7	3	26·7	26·7–26·7	27·0[1]; 26·6[4]
" " " (2)	—	4	26·1	26·0–26·2	26·2[1]
Chlormerodrin B.P.C.	54·6	4	54·5	54·4–54·5	54·6[1]
Tolylmercuric acetate (1) ..	57·2	4	60·5	60·4–60·6	60·8; 60·9[2] 61·9[3]
" " (2) ..	—	2	61·5	61·4–61·6	62·0[2] 63·0; 63·3[3]
" " (3) ..	—	2	61·4	61·3–61·4	61·8[2]
Ethylmercuric chloride (1) ..	75·7	3	71·7	71·5–72·0	74·5; 72·4 72·3[2]
" " (2) ..	—	3	74·7	74·5–75·0	75·6; 74·9[2]
" " (3) ..	—	4	75·1	75·0–75·2	75·1[2] 74·2[4]
Phenylmercuric *p*-hydroxybenzoate	48·4	5	45·8	45·1–46·1	45·7; 45·3[2]
Phenylmercuric acetate	59·6	4	59·4	59·2–59·5	59·6; 59·5[2] 59·6[3]
Phenylmercuric chloride ..	64·1	4	64·5	64·4–64·6	64·0[2]
Ethylmercuric phosphate ..	72·6	4	71·2	71·2–71·3	73·0; 72·0[2]
Ethylmercuric methyl phenyl dithiocarbamate	48·7	3	52·1	52·0–52·2	52·0[2]

[1] Official method of the B.P. or B.P.C.
[2] Sulphide precipitation after destruction of organic matter with hydrogen peroxide and sulphuric acid. Method of Tabern and Shelberg (1932).
[3] Titration with thiocyanate after destruction as in 2. Method of Tabern and Shelberg (1932).
[4] Method of Brookes and Solomon (1959).

The second method was preferred since it is specific for mercury and needs only one standard solution which can be prepared from pure zinc.

The recommended reagents and general method are as follows.

Reagents

Bromine water. A saturated solution of bromine in water. EDTA *solution*, 0·005M. A solution of disodium ethylenediamine tetra-acetate (approximately 1·86 g.) in water (1 litre). This solution need not be accurately standardised. *Zinc solution*, 0·01M. A solution containing pure zinc (0·6538 g.) dissolved in the minimum quantity of hydrochloric

TABLE III

RESULTS OBTAINED ON VARIOUS PILL AND TABLET FORMULATIONS

Preparation	Mercury content					
	Calculated from formula	By flask method			By alternative method	Results expressed as
		No. of detns.	Mean result	Range		
Tab. Calomel B.P.C.	0·25	4	0·237	0·234–0·238	0·24[1]	gr. HgCl per tablet
Tab. Calomel, Rhubarb and Colocynth Co.	2·0	4	1·99	1·96–2·01	1·92[2]	gr. HgCl per tablet
Tab. Calomel, Jalap and Santonin	1·33	4	1·28	1·28–1·28	1·30[2]	gr. HgCl per tablet
Tab. Mersalyl Co.	31·8	4	29·6	29·4–29·7	30·2[2]	mg. Hg per tablet
Pil. Calomel and Colocynth B.P.C. 1949	63·0	4	62·0	61·5–62·5	60·9[1]	mg. HgCl per pill
Pil. Calomel, Colocynth and Hyoscyamus B.P.C. 1949 ..	63·0	4	60·6	59·4–61·8	60·9[1]	mg. HgCl per pill
Pil. Mercury B.P. 1948 ..	1·33	3	1·37	1·37–1·38	1·33[1]	gr. Hg per pill
Pil. Calomel and Menthol ..	0·25	3	0·225	0·223–0·226	0·218; 0·214[3] 0·224; 0·223	gr. HgCl per pill
Pil. Red Mercuric Iodide ..	0·0625	3	0·058	0·057–0·058	0·058; 0·055[4]	gr. HgI_2 per pill

[1] The official method of the B.P. or B.P.C.
[2] The method described for Pills of Calomel, Colocynth and Hyoscyamus in the B.P.C. 1949.
[3] The method for Tab. Calomel B.P.C.
[4] The method for Mercuric Iodide B.P.C. 1949.

acid and diluted to 1 litre with water. *Ammonia buffer solution.* Ammonium chloride (13·5 g.) in strong solution of ammonia (114 ml.) diluted to 200 ml. with water. *Potassium iodide.* PAN *indicator solution.* A 0·1 per cent solution of 1-(2-pyridylazo)2-naphthol in ethanol (95 per cent). This solution is stable for several weeks.

Method

Accurately weigh a suitable quantity of the sample containing about 25 mg. of mercury, transfer to a strip of filter paper (Whatman No. 1 of a suitable size according to the sample) and burn by the method previously described by Johnson and Vickers (1959). Use bromine

solution (5 ml.) in water (20 ml.) as the absorbing liquid. When combustion is complete shake the flask vigorously for about 5 min., and then open in the usual manner. Remove the excess bromine from the solution by drawing a stream of air through it until colourless and then for a further 5 min. Rinse the aspiration tube with a little distilled water, and add 0·005M EDTA solution (30 ml.), 5 ml. of ammonia buffer solution and 0·3 ml. PAN indicator solution to the flask. Titrate with 0·01M zinc solution to the first pink colour using a magnetic stirrer. Add potassium iodide (2 g.), stir the solution for 2 min., and continue the

TABLE IV

RESULTS OBTAINED ON VARIOUS OINTMENTS

Preparation	Mercury content per cent				
	Calculated from formula	By flask method			By alternative method
		No. of detns.	Mean result	Range	
Ointment Mercury Strong B.P.C.	30·0	4	30·2	30·2–30·2	28·9[1]
Ointment Mercury Compound B.P.C. (1)	12·0	4	12·4	12·4–12·5	11·7; 12·0[1]
" " (2)	—	3	12·7	12·5–12·8	12·4[1]
Ointment Mercury Dilute B.P.C.	10·0	4	9·72	9·67–9·74	9·55; 9·40[1]
Ointment Mercuric Nitrate, Strong B.P.C.	not less than 6·7	4	7·06	7·02–7·08	6·92[1]
Oleated Mercury B.P.C.	20·0 per cent HgO	4	20·6	20·6–20·7	20·4[1] per cent HgO

[1] Official method of the B.P. or B.P.C.

titration, again to the pink colour. Each ml of 0·01M zinc solution required after addition of the potassium iodide is equivalent to 0·0020061 g. of Hg.

With ointments, the sample is weighed on to a small square of greaseproof paper which is folded so as to completely enclose the material: this is then itself folded in filter paper as usual.

RESULTS AND DISCUSSION

The method was first applied to phenylmercuric acetate of microanalytical reagent grade (theoretical mercury content 59·57 per cent) and gave satisfactory recoveries (Table I). Table II lists results obtained on some organc-mercury compounds used in pharmacy and agriculture, both by the proposed flask combustion method and other methods. Table III shows the results obtained on a number of pill and tablet formulations and Table IV those obtained on ointments. The method described is applicable only to materials with a mercury content $\geqslant$ 5 per cent: attempts are being made to extend the method to materials of lower mercury content.

It was necessary to investigate whether some alloying of mercury and platinum could occur during combustion. A spiral of silica was used as a sample holder and assays were again carried out on phenylmercuric

acetate (micro-analytical reagent grade). The results obtained were identical with those using the conventional platinum holder (Table I).

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The paper was presented by MR. VICKERS.

4-ALKOXYPIPERIDINES RELATED TO REVERSED ESTERS OF PETHIDINE

BY A. F. CASY AND A. H. BECKETT

From the School of Pharmacy, Chelsea College of Science and Technology, London S.W.3

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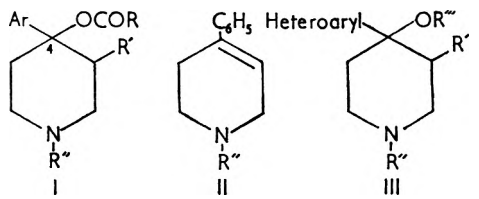
IN reversed esters of pethidine (I) the ester function at C(4) is important for analgesic activity as the corresponding alcohols are inactive. Since hydrolysis has been established as a major metabolic pathway in such



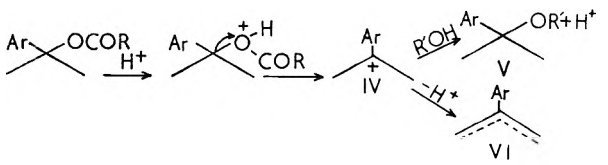
I II III

compounds as pethidine, anileridine and ethoheptazine (Way and Adler, 1960), species differences in activity may, in part, arise from differences in enzyme hydrolysis rates. It follows that introduction of factors that hinder hydrolysis may result in esters that have high activity.

One method of slowing hydrolysis is to introduce steric factors in the vicinity of the ester function. This is illustrated by the work of Levine (1955) on ester hydrolysis in human serum and by work on acetylcholine analogues (Beckett, Harper and Clitherow, 1961; Thomas and Stoker, 1961). The steric influence of an *ortho* methyl group in 4-phenylpiperidines is well demonstrated by its interference with the conjugation of 4-phenyltetrahydropyridines (II) (Fullerton, 1960). The same group might be expected to impede the hydrolysis of the corresponding 4-*o*-tolyl esters and this retardation could result in such esters having analgesic potencies greater than those of their less hindered counterparts. The ester [I, R=R'=Me; R''=$(CH_2)_2$Ph; Ar=*o*-tolyl] is, in fact, a highly active analgesic in mice with a potency greater than that of its 4-phenyl analogue (Beckett, Casy and Kirk, 1959). Keats, Telford and Kurosu (1960) found the same 4-*o*-tolyl ester to be 3–4 times more potent than morphine against post-operative pain in man.

During the syntheses of some heteroaryl counterparts of such reversed esters (I, Ar=Heteroaryl) it was found that certain members could be readily converted, with acid, into the 4-alkoxy analogues (III, R'''=alkyl), some of which possessed significant analgesic activity in mice. Some flexibility in the structure of the oxygen function at C(4) is already evident in pethidine and its reversed esters, and the present work has shown that an ether function at this position may also satisfy structural requirements for analgesia. Since ethers are more stable than esters and probably

less prone to enzyme attack, their substitution for ester groups represents another potential means of retarding metabolic deactivation of piperidine analgesics.



Transformation of esters into the corresponding ethers is considered to proceed via carbonium ions generated by acid-catalysed alkyl-oxygen fission of the esters (Casy, Beckett and Armstrong, 1961). The ions further react either with an alcohol (serving as a nucleophile) giving ethers (V) or by proton loss giving olefins (VI). In some instances the latter have been isolated together with the ethers.

The generation of carbonium ions and their fate depend in large measure upon the electronic nature of the 4-aryl substituent. Carbonium ion formation is facilitated by C(4)-substituents of high electron releasing power [e.g., 4-(*p*-methoxylphenyl) and 2-furyl]. Esters substituted with groups of poorer electron releasing power (e.g., phenyl, *p*-tolyl) or of electron withdrawing character (e.g., 2-pyridyl) are stable under the same acid conditions.

The fate of the carbonium ion depends upon the size and nature of the nucleophilic reagent: ethers result from unbranched alcohols, olefins from their branched isomers. The corresponding ethers would display considerable steric hindrance and their formation is thus contraindicated. Electronegative substituents in the β-position of the attacking reagent do not affect the reaction path, for example, both ethanol and β-chloroethanol give ethers. With allyl alcohol, on the other hand, the olefin results whilst with n-propanol, which is similar in size, the n-propoxy ether is obtained.

A series of 4-alkoxypiperidines (III) have been tested for their analgesic properties in mice and a number found to be more active than morphine (Casy, Beckett, Hall and Vallance, 1961). One of the more active members [III, Heteroaryl=2-furyl; R′=Me; R″=$(CH_2)_2$Ph; R‴=Et] has been examined in detail in mice and shown to be a true morphine-type analgesic from the following results:

(1) It is active in both the hot plate and tail pinch procedures;

(2) Its analgesic properties are antagonised by small amounts of nalorphine;

(3) Mice develop tolerance to its analgesic effects;

(4) It produces mydriasis at low dose levels;

(5) It gives a positive Straub tail effect at low dose levels and has a Straub Index (Shemano and Wendel, 1960) similar to that of morphine indicating the two compounds have similar degrees of addiction liability.

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The paper was presented by DR. CASY.

A SPECIFIC SOLVENT-EXTRACTION METHOD FOR THE DETERMINATION OF TRACE QUANTITIES OF FLUORIDE

By C. A. Johnson and M. A. Leonard

From Analytical Development Group, Standards Department, Boots Pure Drug Co. Ltd., Station Street, Nottingham

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A method is described for the specific determination of trace quantities of fluoride. The procedure depends upon the solvent extraction and subsequent colorimetric measurement of the blue complex formed between fluoride and the cerous-alizarin complexan chelate. Concentrations of 0·25 to 7 μg. of fluoride in 150 ml. of water can be determined. The method has been shown to be applicable to distillates from strong acid and has been used for solid samples of low fluoride content.

Present methods for the determination of trace quantities of fluoride depend upon non-specific reactions with dyestuff-metal chelates. Examples are the thorium-alizarin S or zirconium-Eriochrome-cyanine R systems described by Snell (1959). They are capable of high sensitivity but do not distinguish between fluoride and other complex-forming ions such as phosphate, sulphate, citrate or oxalate.

The method from which we have developed the present technique makes use of a specific and positive reaction between fluoride ions and the cerous chelate of the dyestuff alizarin complexan (3-aminomethylalizarin-*NN*-diacetic acid) first described as a spot-test by Belcher, Leonard and West (1959). This has been applied to the quantitative determination of fluorine in organic compounds on the submicro (Belcher, Leonard and West, 1959) and semi micro scales (Johnson and Leonard, 1961).

Alizarin complexan, due principally to the powerful nitrogen diacetic acid chelating centre, forms a highly associated red complex with cerous ions at pH 4·0 to 4·6. In the presence of low concentrations of fluoride ions (5 to 200 μg./100 ml.) the usual non-specific bleaching effect exerted on metal-dye chelates does not occur; instead the fluoride ion itself enters the structure to form a blue colour with a different spectrum from that of both the cerous chelate (red) and the free alizarin complexan (yellow). The sensitivity of this reaction in aqueous solution is similar to that of the best bleaching systems. Leonard and West (1960), discussing theoretical aspects of the reaction, drew attention to the possibility of selective extraction of the fluoride complex into an organic solvent system. Such an extraction method should lead to an increase in sensitivity and this we have found to be the case. It is necessary to use an extractant consisting of a dilute solution of a hydrophobic amine in a higher alcohol. The presence of an amine is essential, hence the extraction must be of the ion-association type (Morrison and Freiser, 1957). Many combinations of amine and alcohol will serve, but the most satisfactory in sensitivity, preferential extraction of the fluoride complex, and clean separation is tribenzylamine in a mixture of pentyl and secondary butyl alcohols. The

extraction procedure described below increases the sensitivity of the aqueous reaction ten-fold and allows the accurate determination of 0·25 to 7 μg. of fluoride in 150 ml. of water, i.e., 0·002 p.p.m. to 0·05 p.p.m.

Experimental

Apparatus. 250 ml. separating funnels were used with a large temperature controlled water bath deep enough to allow almost complete immersion of the funnels.

A jacketed distillation apparatus was essentially as described by Huckabay, Welch and Metler (1947) but modified by the addition of a spray trap between the steam generator and the apparatus, and an efficient two-sphere spray trap between the jacket and the downward condenser.

Extinction values were measured in 4·0 cm. cells with a battery operated Unicam S.P. 600 visual range spectrophotometer.

Reagents

Alizarin complexan and cerous nitrate* have the composition described by Johnson and Leonard (1961).

Acetate buffer (pH 4·3) is composed of hydrated sodium acetate (75 g.) glacial acetic acid (75·0 ml.) made up to 1 litre with water.

Standard fluoride solution (1·00 μg./ml.). Dissolve about 22 mg. (accurately weighed) of dried analytical reagent grade sodium fluoride in water and adjust the volume to 1 litre. Dilute an aliquot of this solution containing 1·00 mg. of fluoride (approx. 100 ml.) to 1 litre. Store in a polythene container.

Extraction solution. 0·06 per cent tribenzylamine in 30 : 70 pentanol-s-butanol.

Mix together 600 ml. of reagent grade pentanol and 1,400 ml. reagent grade s-butanol. Weigh out 1·20 g. of tribenzylamine (recrystallised from ethanol if necessary) and dissolve by gentle warming in approximately 100 ml. of the alcohol mixture. Transfer the solution of amine to the bulk of the alcohol mixture and mix well.

Procedure

Preparation of calibration graph. Into each of a series of eight 250 ml. separating funnels place 150–n ml. of distilled water (n = ml. of fluoride solution subsequently added); 0 to 7 ml. of standard fluoride solution (1·00 μg./ml.); 10·0 ml. of alizarin complexan solution and 2·0 ml. of pH 4·3 buffer solution. Mix the solutions and place the separators in a water bath, maintained at 25° ($\pm$0·2°) for 10 min., swirling the solutions periodically. Then add 10·0 ml. of cerous nitrate solution to each separator, mix thoroughly and replace in the bath for a further 10 min., again with occasional swirling. Add 40·0 ml. of the extracting solution, previously adjusted to 25°, to each of the separators in turn, shaking for 15 sec. immediately after each addition. Maintain the contents of the separators at 25° for 1 hr. shaking each for 30 sec. every 10 min. Remove

* Available from Messrs. Hopkin and Williams Ltd.

the separators and allow to stand for 15–20 min. at approximately 20–25°, run off and discard the aqueous layer, shaking the contents of the separator as little as possible. Swirl the organic layers around the interior surface of the separators and allow to stand for 5 min. with occasional slight agitation. Run off any additional aqueous layer, at the same time bringing the liquid interface to the bottom of the stopcock bore. Place small loosely packed plugs of cotton wool in the stems of the separators and pass the alcoholic solutions into 25 ml. graduated flasks. Rinse down the walls of the separators with dehydrated alcohol B.P. reagent and allow the rinsings to drain into the standard flasks until the

TABLE I

EFFECT OF FOREIGN IONS

Ion	Wt. of ion (μg.) causing 10 per cent decrease in the extinction at 580 mμ produced by 4·12 μg. fluoride	Molar ratio interfering ion/fluoride
Citrate	67·5	1·64
Phosphate	26·6	3·96
Carbonate	4,050	311
Perchlorate	6,830	325
Sulphate	10,300	495
Nitrate	11,800	875
Chloride	21,300	2,760
Ferric*	82·2	6·77

* Ferric iron forms a red-brown chelate with alizarin complexan which is extracted into the organic layer and causes an initial increase in extinction value. As the ferric ion concentration increases, however, a progressively dense purple precipitate is formed and the density of the filtered alcohol layer decreases.

mark is reached. Shake the flasks well to dissolve any water droplets and read the extinctions of the solutions against that containing no fluoride in 4·0 cm. cells at 580 mμ, the absorption maximum of the blue complex. Unknown fluoride solutions (150 ml.) are treated similarly.

When a preliminary distillation is necessary, place the sample in the inner bulb of the distillation apparatus and rinse down the walls of the entry tube with as small a volume as possible of distilled water followed by the rapid addition of 25 ml. of 60 per cent w/v sulphuric acid. Connect the steam generator, already heated to a temperature of 60–80°, to the apparatus, replace the stopper and heat the liquid in the jacket (sym. tetrachlorethane) until it refluxes in the condenser. Heat the steam generator to boiling and collect 150 ml. of distillate. Treat this solution for colour development, extraction and measurement as described above.

Significant amounts of fluoride were frequently found in new glass apparatus and several trial runs were then made before estimations began. This agrees with the findings of the Society for Analytical Chemistry Sub-Committee report (1944).

Effect of Foreign Ions

The quantitative effect of some foreign ions has been evaluated. Table I shows the molar ratio of interfering ion necessary to bring about a 10 per cent reduction in the colour produced from 4·12 μg. of fluoride.

Results and Discussion

Calibration graphs prepared as described are linear over the range 0 to 5 μg. of fluoride but at higher concentrations a slight increase in slope is apparent. In the range 0 to 5 μg. the sensitivity is 0·13 extinction unit per μg. of fluoride. The blank value, prepared as described, is high but

TABLE II

Recovery experiments on standard fluoride solutions

Wt. fluoride taken (μg.)	Wt. fluoride found (μg.)	No. of determinations
Without distillation		
1·00	0·99 ± 0·07	5
2·00	1·99 ± 0·04	5
4·12	4·12 ± 0·03	5
5·00	5·07 ± 0·03	7
With distillation		
1·00	1·01 ± 0·06	8
2·00	1·98 ± 0·09	6
5·00	5·16 ± 0·09	7

reasonably reproducible; fifteen separate determinations, accumulated over a period, gave a mean value of 0·399 with a standard deviation of 0·007 when measured against pure ethanol.

The precision of the method was determined by applying the procedure to known volumes of standard fluoride solution. Table II lists the results obtained.

Initially, distillation recoveries were made using the more usual 60 per cent perchloric acid rather than sulphuric acid but low values were

TABLE III

Results obtained on some solid materials

Sample	Wt. taken (g.)	Wt. fluoride added (μg.)	Wt. fluoride found (μg.)	Fluoride content of sample (p.p.m.)	Fluoride recovered (μg.)
Potassium hydrogen phosphate (Analar) ..	5·00	0	1·11	0·22	—
	5·00	0	1·06	0·21	—
	5·00	2·00	3·38	—	2·29
	5·00	2·00	3·26	—	2·17
Calcium phosphate	0·1085	0	2·01	18·5	—
	0·1215	0	2·34	19·2	—
	0·0892	2·00	3·51	—	1·83
	0·0948	2·00	3·78	—	1·99
Baking powder (previously ashed with 1 ml. 10 per cent sodium carbonate before distillation)	0·6173	—	2·90	4·7	—
	1·1560	—	4·50	3·9	—
	0·8880	—	4·17	4·7	—
	0·8491	—	3·82	4·5	—
	0·8307	—	3·74	4·5	—
	0·7280	—	2·77	3·8	—

obtained. These were shown to be due to the presence of perchlorate ions in the distillate.

Table III lists results obtained on samples for which trace fluoride determinations are commonly required. The sample of calcium phosphate was a reagent grade material, as opposed to bone phosphates which usually contain much higher proportions of fluoride.

Distillations in the absence of sample gave low and fairly reproducible blanks of 0·19 ± 0·06 μg. fluoride.

Where samples of fluoride in concentrations greater than 5 μg. in 50 ml. of water and free from all interfering ions, are available, the thorium-alizarin titration procedure is applicable if a titrant as dilute as 0·00025M is used. Such a method is more rapid than the present one and is capable of similar precision: sulphate and phosphate, however, produce gross interference. The method described is of value where low concentrations of fluoride are present or where only small quantities of sample are available such as in the analysis of human blood serum. It is far less susceptible to interference than the titration method and is specific for fluoride.

In addition to the types of sample discussed, it seems probable that the method would be of value for effluent and atmospheric pollution studies.

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The blue colour was formed between 1 and 200 μg. of fluoride per 100 ml.; over 500 μg. the bleaching effect of the fluoride began to interfere. The limiting factor was mainly the variation in density readings of the spectrophotometer. Interference from cyanide was negligible. Temperature control was important. The alizarin complex had a zwitterion arrangement similar to that of the EDTA molecule.

ENTRAINMENT AND FLOODING IN VERTICAL STILL-HEADS

BY A. J. EVANS, E. SHOTTON AND D. TRAIN

*From the Department of Pharmaceutics, School of Pharmacy, University of London, Brunswick Square, London, W.C.*1

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An investigation has been made of the entrainment entering, and collected on, vertical still-heads from a boiling solution. The entrainment entering the still-heads increased gradually with increasing distillation rate until the Reynolds Number of the vapour in the still head reached a value of about 10,000. At this point a much greater rate of increase was noted and was accompanied by a greater rate of collection of the entrainment over all sections of the still-head. The value of 10,000 for the Reynolds Number was verified from the observations of others. The true flooding effect did not occur until much higher distillation rates, when there was a sudden increase in the volume of liquid collected at the top of the still-head and a corresponding decrease in that collected at the bottom. Increasing the length of the still-head from 30 to 60 inches showed an increase in maximum collecting efficiency from approximately 96 to 99 per cent. Decontamination factors compared favourably with those obtained by other workers using more complex packed columns.

WHEN boiling any liquid, it is invariably found that the vapour immediately above the liquid surface contains varying proportions of droplets of the original liquid. The carriage of these droplets by the vapour stream is now generally referred to as entrainment, and it is in this context that the word is used throughout this paper.

The work was an extension of that of Shotton and Habeeb (1954) and Train and Velasquez-Guerrero (1957). They were concerned with the estimation of entrainment which had passed through a still-head of known characteristics, and had been collected in a centrifugal separator. Habeeb (1954) made an attempt to collect the liquid caught on the walls of the still-head but the results were found to be variable and could not be related to the distillation rate. This variability was due to an inadequate trapping channel, and to the design of the apparatus which allowed some direct splashing of the boiler liquid to a point above the channel.

Garner, Ellis and Lacey's work (1954), on the other hand, involved mainly an estimation of size and number of entrained liquid droplets. It is difficult, if not impossible, to give a full estimate of the total entrainment, since samples cannot be obtained from immediately above the surface of the boiling liquid. The higher the sampling level above the boiling liquid surface, the smaller will be the estimate of "total" entrainment, since a certain proportion of the droplets projected from the surface will fall back into the boiling liquid.

The principal aims of this work therefore were to study the "total" entrainment passing into given still-heads at various distillation rates and to determine the proportions collected over various sections of the still-head, together with that which passed through to a centrifugal separator.

EXPERIMENTAL

Apparatus

The design of the apparatus was based on that used by Shotton and Habeeb (1954) and a flow diagram is given in Fig. 1. The capacity was much increased so that conditions resembled those obtained in an industrial pilot-scale plant. Higher steam rates were used to cover a complete range of flow from the streamline conditions referred to by Shotton and Habeeb (1954), through the turbulent region to a point equivalent to full flooding conditions in a wetted-wall column.

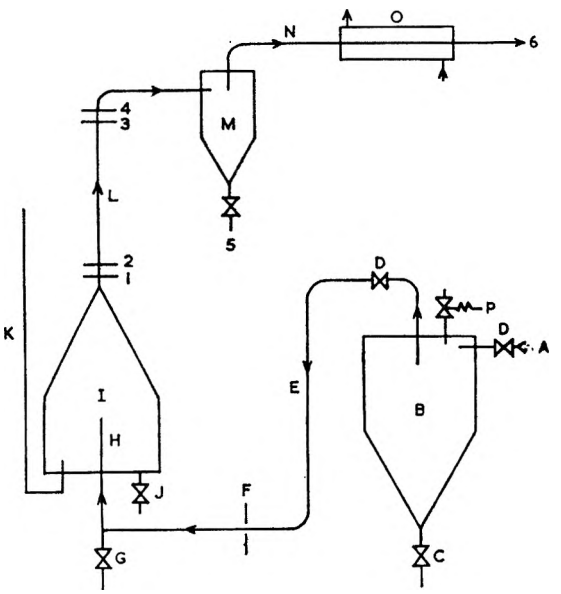

FIG. 1. Flow diagram of apparatus.

A — ¾ in. steam supply.
B — Expansion vessel.
C — Drain valve and steam trap.
D — Control valves.
E — 2 in. steam line.
F — 1 in. orifice.
G — Drain valve.
H — Steam injector.
I — Cone and boiler.
J — Drain valve.
K — Filling tube and level indicator.
L — Still-head.
M — Centrifugal separator.
N — 2 in. vapour line.
O — Tube and shell condenser.
P — Safety valve.
1, 2, 3, 4, 5 — Entrainment collecting points.
6 — Condensate.

The boiler was of stainless steel with a total capacity of approximately 20 l. Vapour passed up through a cone, forming the upper part of the still into a 2 in. diameter still-head without obstruction to its free flow. At the base of the still-head were situated two collecting rings which were integrated in what is termed a collecting unit (Fig. 2). This unit was so designed that the lower ring S (collecting point 1, Fig. 1) collected any liquid rising up the wall of the cone and prevented its further passage up the still-head. The upper ring T (collecting point 2) was intended to collect any entrainment plus condensate caught on the walls of the still-head, which subsequently drained downwards, under the influence of

gravity. This unit was designed to facilitate liquid collection without interfering with the flow pattern of the continuous steam phase.

A similar collecting unit was inserted at the top of the still-head, the lower part (collecting point 3) to trap any climbing film from the still-head and the upper part (collecting point 4) for entrainment plus condensate caught on and near the close angle bend just above it which would normally have drained back into the still-head. This general arrangement enabled trapping areas to be defined, and also showed whether liquid on the wall was moving up or down the still-head, under given flow conditions.

Two metal still-heads were used, giving a distance between the centres of the collecting units of 30 in. or 60 in. respectively; in addition a 30 in

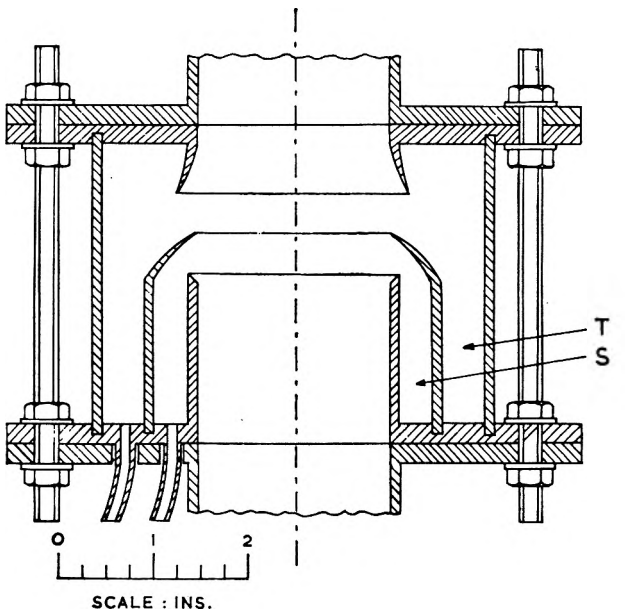

FIG. 2. Still-head collecting unit.

Pyrex glass still-head of the same diameter was also used to enable visual observations to be made. Experiments were also made with the close angle bend connected directly to the lower collecting unit.

Steam was introduced into the boiler using a steam sparger designed to eliminate violent bubbling action of the entering steam and to prevent loss of boiler contents when the steam was not flowing. The bulk of the apparatus, consisting of the steam sparger, cone, still-heads, collecting units, separator and their connecting pipes, was given a dull chrome finish to avoid contamination of the fluids contained in them, and to provide a surface which could be completely wetted. The apparatus was lagged to reduce the condensation, although it was appreciated that some condensate was necessary in order that efficient collection of the entrained droplets could be ensured.

Procedure

The method of estimation of entrainment was essentially the same as that used by Shotton and Habeeb (1954) and by Train and Velasquez-Guerrero (1957). This consisted of the use of a boiler solution containing 0·1 per cent (≡1,000 μg./ml.) of Fluorescein Sodium B.P. By this means droplets of original solution, which were entrained and subsequently

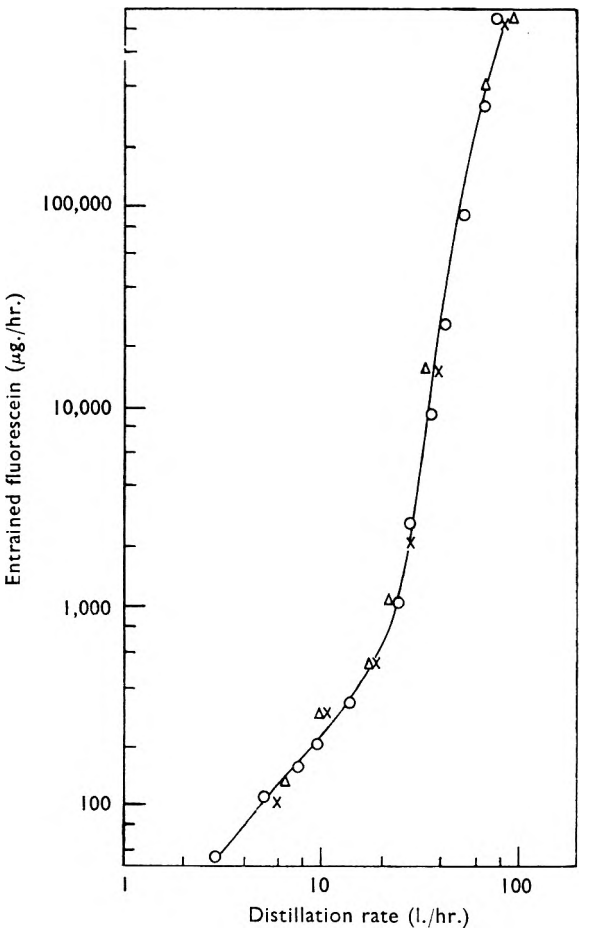

FIG. 3. "Total" entrainment collected—from points 2, 3, 4, 5 and 6.
× "Blank" still-head.
○ 30 in. still-head.
△ 60 in. still-head.

collected over various sections of the apparatus, would mix with any condensate collected from the same section, and form a comparatively dilute solution, on which a quantitative estimate of fluorescein content could be made by means of a Spekker Fluorimeter. This method of estimation has been fully described by Shotton and Habeeb (1955).

The still was initially heated by two 1 kilowatt heating elements for approximately 30 min. or until the solution was boiling, the apparatus warmed through and some condensate obtained. The liquid in the boiler was then readjusted to a predetermined level, the heaters switched off, and the steam introduced at the required rate. 30 min. was allowed for the

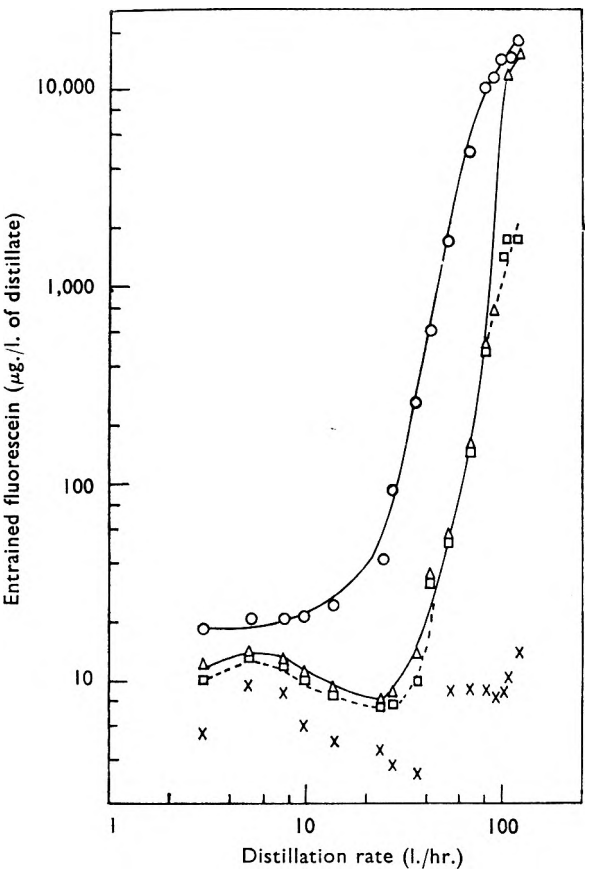

FIG. 4. Entrainment collected from succeeding sections of 30 in. still-head.
○ From points 2 + 3 + 4 + 5 + 6.
△ From points 3 + 4 + 5 + 6.
□ From points 5 + 6.
X From point 6.

system to reach equilibrium and an experimental run made during the succeeding 30 min. At the end of a run the solutions from the five collecting points and the condensate were measured and retained for fluorimetric estimation, and the liquid level in the still checked.

Entrainment was calculated as the total amount of fluorescein collected over the various parts of the still-head in μg./hr. All figures for entrained fluorescein were related to a constant concentration of 1,000 μg./ml. in the still throughout each individual experiment (Evans, 1961).

The rate of distillation was determined from the sum of the volumes of solution collected at points 2, 3, 4 and 5, together with the final distillate. Solution collected from point 1 was not included, since, particularly at the high rates of distillation, the bulk of it entered the collecting duct as a result of a climbing film effect up the cone from the boiling solution, and not as true condensate.

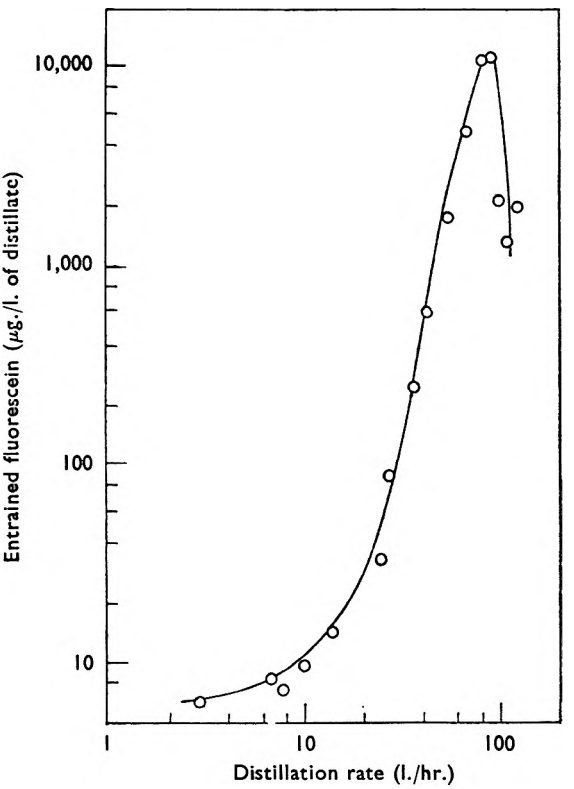

FIG. 5. Entrainment collected from bottom of 30 in. still-head (Point 2).

Quantities of liquid held on the still-head surface at distillation rates near the flooding point were determined by rapidly shutting off the steam supply and collecting the liquid draining down the still-head.

RESULTS

Entrainment entering the three still-heads over the full flow range used in the experiments is shown in Fig. 3, showing that the entrainment passing from the boiler into the various still-heads was constant for a given rate of distillation. The entrainment passing through to succeeding sections of the apparatus is exemplified by the results shown in Fig. 4 using the 30 in. still-head. Detailed results of other lengths are available (Evans, 1961). The entrainment collected on any particular section is

represented by the vertical height between adjoining plots, and the amount falling down the still-head surface is shown in Fig. 5. The changes in mass movement of liquid on the wall of the still-head are tabulated in Table I, and the movement could also be seen in the glass still-head, corresponding to the normal flooding conditions in a wetted-wall column.

TABLE I

VOLUMES COLLECTED ON 30 IN. STILL-HEAD

Distillation rate (l./hr.)	Collecting points		Reynolds Number of vapour
	2 (ml./hr.)	3 (ml./hr.)	
2·9	81	24	1,617
13·86	76	13	7,720
24·08	77	16	13,420
35·75	87	18	19,920
53·01	174	31	29,500
68·32	390	33	38,050
82·15	920	36	45,750
89·9	988	39	50,050
91·16	1,074	30	50,800
97·4	532*	568	54,300
101·1	300	1,052	56,300
107·82	216	1,116	60,500

* Hold-up volume of liquid on still-heads at flooding rate = 9·7 ml.

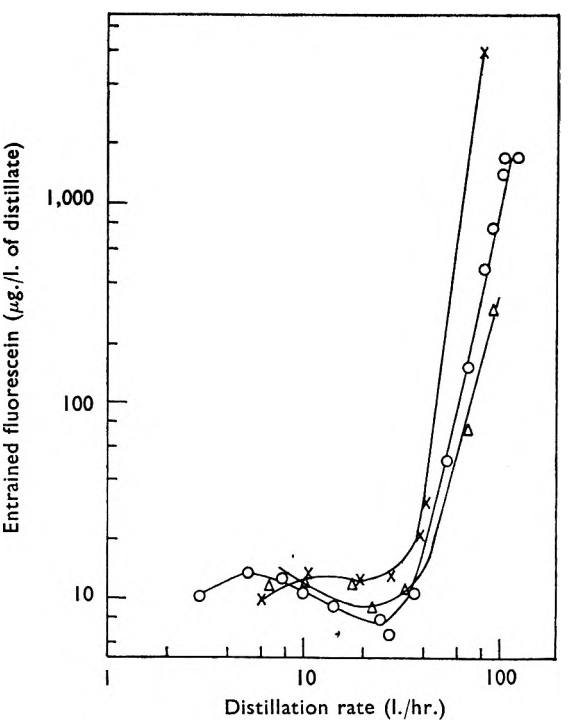

FIG. 6. Entrainment passing through still-heads.
X "Blank" still-head.
○ 30 in. still-head.
△ 60 in. still-head.

An example of the hold-up volumes of liquid held on the still-head at the flooding rate is given as a footnote to Table I.

It was noticed in the glass still-head at distillation rates of about 20 litre/hr. that there was a wave motion on the surface of the liquid film on the wall; the wave motion ascended the column against the downward flow of the liquid due to gravity.

The entrainment passing through the various length still-heads is shown in Fig. 6.

Discussion

This work has shown that the total entrainment entering three different length still-heads is substantially the same for a given rate of distillation

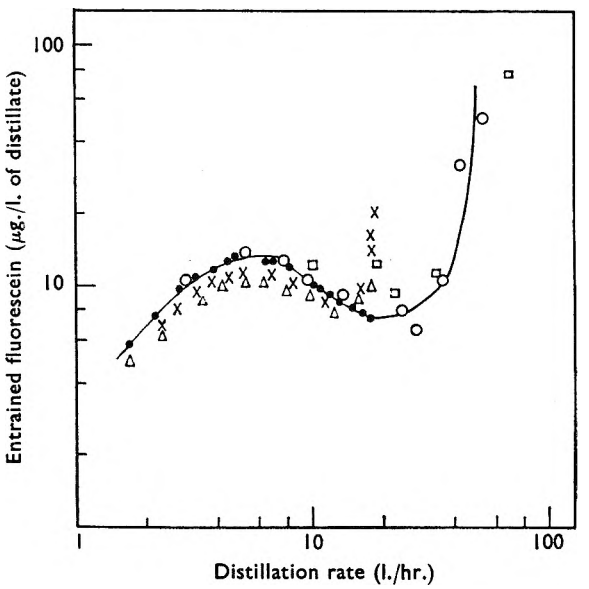

Fig. 7. Correlation of results of entrainment passing through various still-heads.

× 10 in. still-head
△ 15 in. still-head
● 30 in. still-head
} Shotton and Habeeb (1954).

○ 30 in. still-head
□ 60 in. still-head
} This work.

(Fig. 3), thus verifying the assumption of both Shotton and Habeeb (1954) and Train and Velasquez-Guerrero (1957). The plot of the entrainment from points 5 and 6 (Fig. 1) for the 30 in. still-head is similar to that obtained by Shotton and Habeeb (1954) for their 30 in. × 2 in. still-head, even though the apparatus differed. A direct comparison of these results is given in Fig. 7. The comparison could not be drawn directly from the figures for entrainment collected in the centrifugal separators, since at low distillation rates the separator used in this work was not working at its maximum efficiency and a certain proportion passed into the condensate.

The entrainment passing over into the separator and condenser for all three still-heads is shown in Fig. 6, and it may be seen that the entrainment per unit volume of distillate passing through the still-head decreased within the range 10–30 litre/hr. It has already been shown in Fig. 3, however, that the "total" entrainment per unit time continually increases, and as a corollary to this the entrainment collected in unit time from the still-head, that is, the summation from points 2 and 3 (Fig. 1), also continually increased.

It may be seen from Fig. 6 that the entrainment passing completely through the 60 in. still-head is greater than that through the 30 in. below a distillation rate of 35 litre/hr. This apparent anomaly is similar to that reported by Shotton and Habeeb (1954), who found that although, in general, entrainment passing through the still-heads decreased with increasing length, the material passing through their 30 in. still-head was greater than that passing through shorter length still-heads over lower ranges of distillation rates.

These apparent anomalies in both this and Shotton and Habeeb's work are thought to be due to the development of a turbulent boundary layer of flowing steam at the upper ends of the longer still-heads. In the presence of turbulent eddies of vapour, any entrained droplets are liable to be given a radial component of velocity and thus would proceed in the general direction of the still-head wall. Only an opposing eddy would alter this radial direction and if, therefore, the droplet entered a streamline boundary layer it would continue along a path approximate normal to axial flow and thus be caught on the walls of the still-head.

As full turbulent conditions are progressively developed in the tube and the annular streamline boundary layer is reduced to the buffer and laminar sub-layers, there is the increasing possibility of the droplets being deflected by further eddies which effectively hold them within the main vapour stream, and therefore reduce their chance of being caught on the walls. The presence of a turbulent boundary layer may also result in the re-entrainment of previously collected droplets because of the turbulent action of the flowing steam on the boundary layers of *liquid* on the walls of the still-head.

It has been shown by both Goldstein (1938) and Coulson and Richardson (1955a), in their textbooks, that a transition takes place at some critical value of the Reynolds number involving the distance from the leading edge. This modified Reynolds number is usually denoted by Re_x, where the length factor x is the distance from the leading edge (in this example the entry point into the still-head) and x replaces the more usual diameter dimension.

The critical value of Re_x for the development of a turbulent boundary layer is usually considered to be about 10^5, although values as high as $1{\cdot}1 \times 10^6$ have been observed, depending on the disturbances present and on the conditions of entry at the leading edge.

It may be expected, therefore, that the longer the still-head the greater the chance of the development of a turbulent boundary layer resulting in the re-entrainment of liquid as described above.

When considering the visual observations, in the glass still-head the liquid caught on the walls did not immediately drain down, but quite large volumes oscillated over several centimetres. The effect of this oscillation, plus any wave formation on the surface, would greatly enhance the possibility of re-entrainment in this region.

"*Climbing Film Effect*"

To avoid any discrepancies due to variation in the quantity of total entrainment entering the still-head, the efficiency of collection of the three still-heads was represented by Fig. 8. The efficiency was approximately

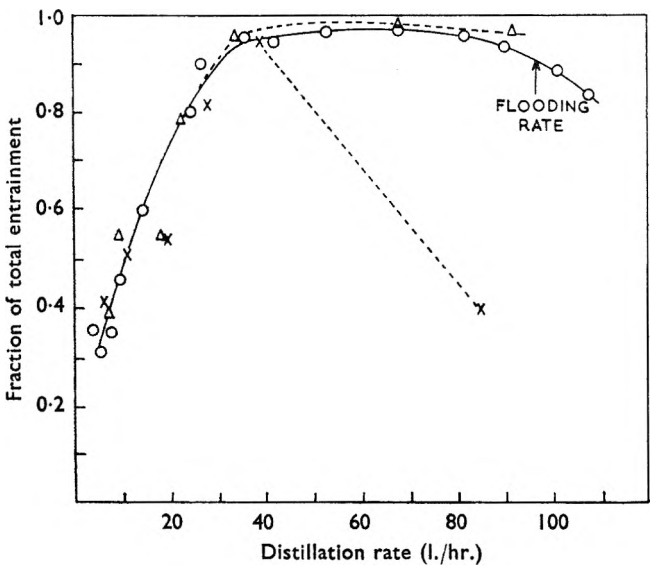

FIG 8. Fraction of "total" entrainment caught on walls of still-head.
X "Blank" still-head.
○ 30 in. still-head.
△ 60 in. still-head.

the same in all still-heads for distillation rates up to 20–30 litre/hr. Just before the onset of flooding conditions within the still-heads, the efficiency of collection begins to decrease. Since the onset of flooding indicates that the vapour flowing through the still-head is capable of exerting sufficient drag force on the liquid on the walls to reverse its direction completely, it is probable that this reduction in efficiency is a result of re-entrainment of some of the collected liquid into the vapour stream. The apparent sudden decrease in efficiency of the "blank" still-head is not strictly comparable with the results from the other still-heads because only one set of collecting ducts was used, corresponding to points 1 and 4, and liquid which was either re-entrained or reversed in its direction of flow would be passing directly into the centrifugal separator (point 5, Fig. 1).

The sudden increase in entrainment collected at points 5 and 6 (Fig. 5) can be compared with the increases found by Shotton and Habeeb (1954) and Train and Velasquez-Guerrero (1957). They found that in all sizes

of still-head they used, the increase in entrainment passing through the still-heads began when the distillation rate corresponded to a value of the Reynolds number of the vapour in the tube in the range 9,000–10,000 (Habeeb, 1954, p. 82). In our work the increase began at distillation rate equivalent to an *Re* value of approximately 11,000. These previous workers referred to the increase in entrainment collection in the separator as the beginning of a climbing film effect or gross carry-over of fluorescein solution.

Our results show that the "climbing film effect" was not present at these distillation rates, and that mass movement of liquid on the wall of the still-head was still downwards (Table I). The apparent "climbing film effect" is due to wave motion on the surface of the liquid film on the

TABLE II

VELOCITIES AT BOILER SURFACES AND IN STILL-HEADS IN THIS AND COMPARATIVE WORK AT POINTS OF SUDDEN INCREASE IN ENTRAINMENT

Reference	Diameter of boiler surface (in.)	Velocity at boiler surface (cm./sec.)	Diameter of still-head (in.)	Velocity in still-head (cm./sec.)	Reynolds Number of vapour in still-head
This work	12	12·7	2	459	11,140
Garner, Ellis and Lacey (1957) ..	12	72·3	12	72·3	10,500
Shotton and Habeeb (1954) ..	9	18·8	2	381	9,240
	9	14·8	1½	534	9,712
	9	9·6	1	777	9,390
	9	6·15	¾	1,278	9,700

wall, the wave motion ascending the column against the downward flow of the liquid. This phenomena has been reported by other workers (Semenov, 1944, and Thomas and Portalski, 1958).

The sudden increase of entrainment entry into the separator is accompanied by a similar increase in collection of entrainment over all other sections of the still-head. The graph of "total" entrainment against distillation rate (Figs. 3 and 4) summarises these effects and indicates that the initial cause of these increased entrainment collections over different parts of the still-head is due to a sudden increase of total entrainment entering the still-head and not differing conditions within the still-head itself.

Garner, Ellis and Lacey (1954) comment on this phenomenon and have shown that above a certain critical distillation rate increase of entrainment is very rapid. In their work, this sudden increase was more marked in the determination of entrainment entering the vapour line at the top of the vapour space than on results from measurements of "total" entrainment near the surface of the boiling liquid. The critical distillation rate in their work was approximately 250 lb./hr., which, in their 12 in. diameter evaporator, corresponded to a value of *Re* of 10,500. They commented that this sudden increase may be caused by a change in boiling conditions at the liquid surface, but since corresponding points of sudden increase of entrainment have been noticed in two other completely different sets of apparatus using widely differing rates of vapour release from the surface

this would not appear to be the controlling factor (Table II). Garner, Ellis and Lacey also considered the terminal velocities of the various size droplets, but again the sudden increase of entrainment could not be directly related with this or the standard theories of elutriation because of the wide divergencies of flow velocities in the different sets of apparatus, the relevant dimensions of which being included in Table II.

The correlating feature of these various critical distillation rates appears to be a function of the Reynolds number of the vapour stream in the still-head. As a corollary to this, it was realised that the product of the mass flow rate Q (g./sec.) and velocity u (cm./sec.) is also a constant factor for this critical point.

That is $Qu = K(Re)^2$ where $K = \frac{\pi\eta^2}{4\rho}$

and is constant for all sets of apparatus.

It is thought that this product Qu may represent the total force available for lifting the particles into the still. It can be seen that the dimensions of the product are the same as that of a force unit, $\frac{ML}{T^2}$.

Garner, Ellis and Lacey (1957) stated that the bulk of the entrainment was caused by the larger droplets, in spite of the fact that over 95 per cent of the number of droplets collected were below 20 μ in diameter.

There appears to be a critical size range of about 17–20 μ above which the entrainment caused by these larger droplets increases approximately as the square of the diameter of the droplet.

It would follow that there is a possible correlation of these critical size ranges of droplets and the force available from the rising vapour, in that, when the force is sufficient to entrain the larger droplets then the "total" entrainment collected in the still-head and subsequent parts of the apparatus, will show a sudden increase.

Pyott, Jackson and Huntington (1935) in their investigations on a kerosene-air system showed that a sudden increase in entrainment occurred with increase of velocity at approximately 1·5 ft./sec. of air. Their experiments were made in a 12·5 in. diameter tower at 80° F. and thus the corresponding value of Reynolds number at the critical point is found to be approximately 7,500. Sherwood and Jenny (1935) working with a water-air system at 20° and in a 45·7 cm. diameter tower showed a more definite critical point to arise at the same vapour velocity as Pyott and others, but in a larger diameter tower. This corresponds to a Reynolds number of approximately 12,000. O'Connell and Pettyjohn (1946), who investigated liquid carry-over in a horizontal tube evaporator, obtained a figure for the allowable mass velocity to prevent splashing. This corresponded for boiling point conditions at 140° F. to a Reynolds number of 10,100 and for 164° F. to a figure of 15,100.

These values of Reynolds numbers are similar to those in Table II.

The Post-entrainment Stage

The true climbing film or flooding effect was clearly manifest when there was a sudden increase in the volume collected at point 3.

Correlation of these results and those of other workers using wetted-wall columns is almost impossible because values of liquid mass flow rates in these experiments were less than 150 lb./hr. per sq. ft. Without altering the standard distillation system the quantity of liquid on the walls of the still-head cannot be appreciably changed. It is dependent solely on the condensate forming on the still-head, and entrained solution collected at the specific flow rate. At flooding conditions the entrained portion contributes over 90 per cent of the total, and is itself directly dependent on the flow rate. Thus no relation was possible with work such as that of Holmes (1947), who investigated flooding velocities in vertical unpacked tubes using much higher liquid flow rates.

The relation between the momentum of the downcoming liquid and the upward resistance force from the rising steam was established, using the data at the flooding rate given in Table I. From the pipe friction chart of Coulson and Richardson (1955b) it was calculated that the resistance to flow, per unit area of still-head, at the flooding point would be 8·1 dynes/cm^2. The practical figure obtained by considering the momentum of the downcoming fluid was 7·9 dynes/cm^2.

Decontamination Factor

In industrial distillation plants the removal of entrained material is of practical concern and in certain instances, as when dealing with radio-active materials, the decontamination factor must be very high. The decontamination factor is defined as the ratio of the activity/ml. of the still pot solution to the activity/ml. of condensed vapour leaving the evaporator.

In our experiments, although only simple entrainment removal devices were used such as a straight length of vertical tubing and a centrifugal separator, the decontamination factors were about 10^5. This compares favourably with the figures 10^4 to 10^5 obtained by Manowitz, Bretton and Horrigan (1955) for example, who made experiments using a Raschig ring packed tower and a "Thermal Wool Fiber-Fiberglass" packed tower.

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The paper was presented by DR. EVANS.

STUDIES ON BACTERIAL POPULATIONS IN SOLUTIONS OF PHENOLS

PART II. THE INFLUENCE OF CELL-EXUDATE UPON THE SHAPE OF THE SURVIVOR-TIME CURVE

BY H. S. BEAN AND V. WALTERS*

From Chelsea School of Pharmacy, Chelsea College of Science and Technology, Manresa Road, London, S.W.3

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The shape of time-survivor curves for *E. coli* suspended in aqueous solutions of benzylchlorophenol depends on the concentration of the bactericide. In some concentrations the last survivors exhibit unexpected multiplication. The death of the organisms is accompanied by the release of cell-constituents having an ultra-violet absorption maximum at 260 mμ. Growth occurred when *E. coli* exudate was added to aqueous suspensions of *E. coli* whereas in its absence the organisms died. The numbers of viable organisms depended upon the concentration of the added cell exudate. The fate of the last survivors of a suspension of *E. coli* in benzylchlorophenol solution was controlled by re-suspending them in components of the reaction mixture. Removal of cell-exudate produced rapid death and the removal of bactericide led to multiplication of the organisms. It is concluded that cell exudate constituted the nutrient substrate for multiplication of last survivors in an initially bactericidal system. The theories of disinfection and the influence of cell exudate upon the shape of time-survivor curves are discussed.

WASHED *Escherichia coli* suspended in a solution containing 50 μg./ml. benzylchlorophenol in 0·01M phosphate buffer (pH 7·0) unexpectedly multiplied after about 7 hr., by which time the survivors had diminished to less than 0·01 per cent of the initial number (Bean and Walters, 1955). During the ensuing 100 hr. or more, a 50 to 500-fold increase in the viable count occurred, and in replicate experiments the maximal counts reached 0·5 to 1·0 per cent of the initial count. The system contained no nutrient material in the accepted sense. Nevertheless, the marked increase in the viable count indicated the existence of a source of nutrient. We suggested that the observed multiplication could be explained by the few surviving organisms utilising water-soluble constituents released from dead organisms. But it could have been due to the utilisation of the phosphate buffer which contained Na^+ and K^+ mono- and di-hydrogen phosphates and, possibly, traces of organic impurities. Many workers (Allan, Pasley and Pierce (1952); Bigger (1937); Bigger and Nelson (1941, 1943); Butterfield (1929); Garvie (1955); Savage and Wood (1917)) have shown that bacteria can multiply in the presence of only traces of nutrient materials.

The present paper reports further experiments to test the hypothesis that *E. coli* can utilise the exudate from cells of a culture and to ascertain whether multiplication of the last survivors in a suspension can occur in various concentrations of benzylchlorophenol.

* Present Address: Nigerian College of Technology, Ibadan, Nigeria.

Experimental

The test organism (*Escherichia coli* Type 1) and experimental methods were as described previously (Bean and Walters, 1955). The improved capillary-dropping pipette (Cook and Yousef, 1953; Cook 1954) was used for sampling the bacterial suspensions and the viable counts were made by the standard roll-tube technique (Berry and Michaels, 1947; Wilson, 1922; Withell, 1938).

The Viability of Untreated E. coli *in Solutions of Cell Exudate*

A sterile solution of cell exudate (optical density $d_{1\ cm.}$ at 260 mμ = 1·2) was prepared by heating thrice-washed *E. coli* in distilled water at 100°

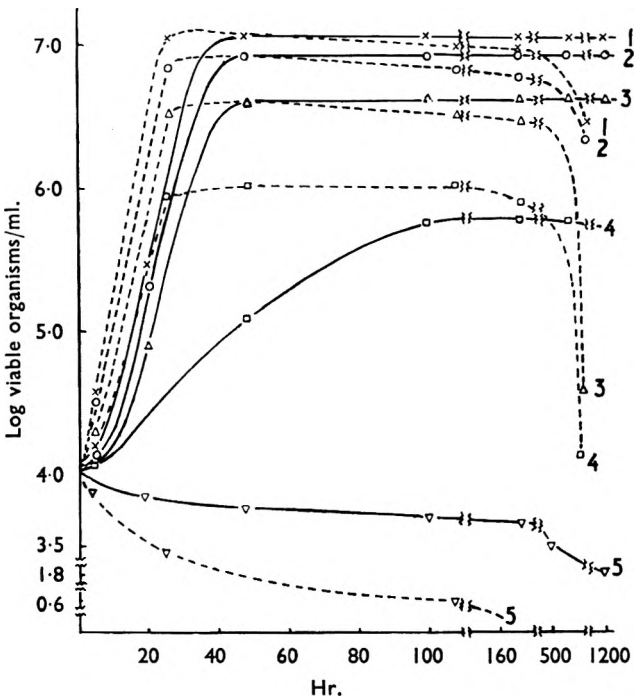

FIG. 1. Effect of concentration of cell exudate solution upon the growth of *E. coli* at 20° C (solid lines) and at 37° (broken lines).
Curves 1–4 represent cell exudate solution having $d_{1cm.}$ at 260 mμ = 1·2, 0·9, 0·6 and 0·3 respectively, curve 5 = distilled water (reference curve).

for 5 min., removing the cells by centrifugation and filtering the supernatant through a sterile 5-on-3 sintered glass filter. The filtrate was diluted with sterile distilled water to give three further solutions containing respectively 75, 50 and 25 per cent of the original exudate solution. To 60 ml. quantities of each of the four solutions and to distilled water as a control, one drop (0·016 ml.) of a diluted washed aqueous suspension of *E. coli* was added. The inoculated solutions were divided into two equal portions, maintained at 20° and 37° respectively, and viable counts were made at intervals.

When suspended in distilled water the organisms died slowly at 20° and much more rapidly at 37° (Fig. 1). In solutions of cell exudate they showed typical growth curves. Each phase of the growth cycle was longer at 20° than at 37°, the optimal temperature for growth (Wilson and Miles, 1955). The increase of number of organisms at the end of the log phase of growth in the respective exudate solutions was about

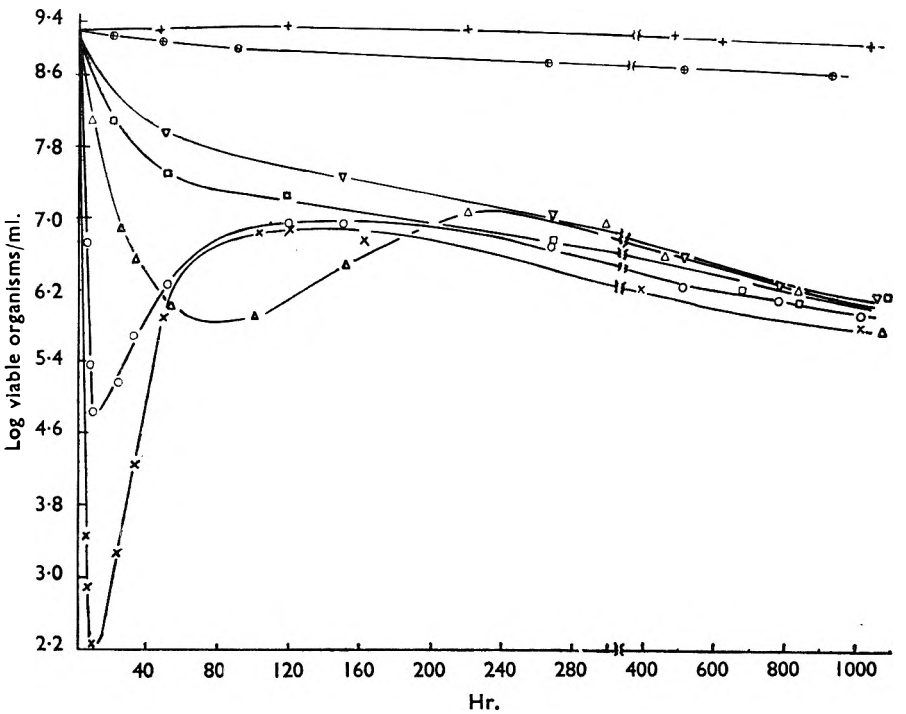

FIG. 2. Time-log survivor curves for *E. coli* in

+——+	cell exudate ($d_{1cm.}$ at 260 mμ = 1·25)	
⊕——⊕	distilled water	
▽——▽	benzylchlorophenol	20 μg./ml.
□——□	"	25 "
△——△	"	27·5 "
○——○	"	33 "
×——×	"	50 "

1,000 fold. It was related to the concentration of the cell exudate solution and was approximately the same at both incubation temperatures.

The optical density at 260 mμ of the cell-free solutions showed that multiplication of the organisms was accompanied by a very small decrease in the concentration of cell exudate. On the assumption that the wet weight of 10×10^6 organisms was 0·01 mg. (Oginsky and Umbreit, 1954) and that each bacterium was composed of 80 per cent water (Wilson and Miles, 1955), then 10×10^6 *E. coli* would have had a dry weight of only 2 μg. Hence, the total mass of organic material synthesised was very small and accounted for the small decrease in cell exudate concentration.

Dead and dying cells would also have contributed some exudate to the solution during the period of the experiment.

The viability of E. coli *in Aqueous Solutions of Benzylchlorophenol*

Phosphate buffer, or impurities therein has been reported to promote the growth of *E. coli* (Garvie, 1955; Chambers, Tabac and Kabler, 1957). To determine whether multiplication of the survivors in solutions of benzylchlorophenol occurs in the absence of the phosphate buffer used

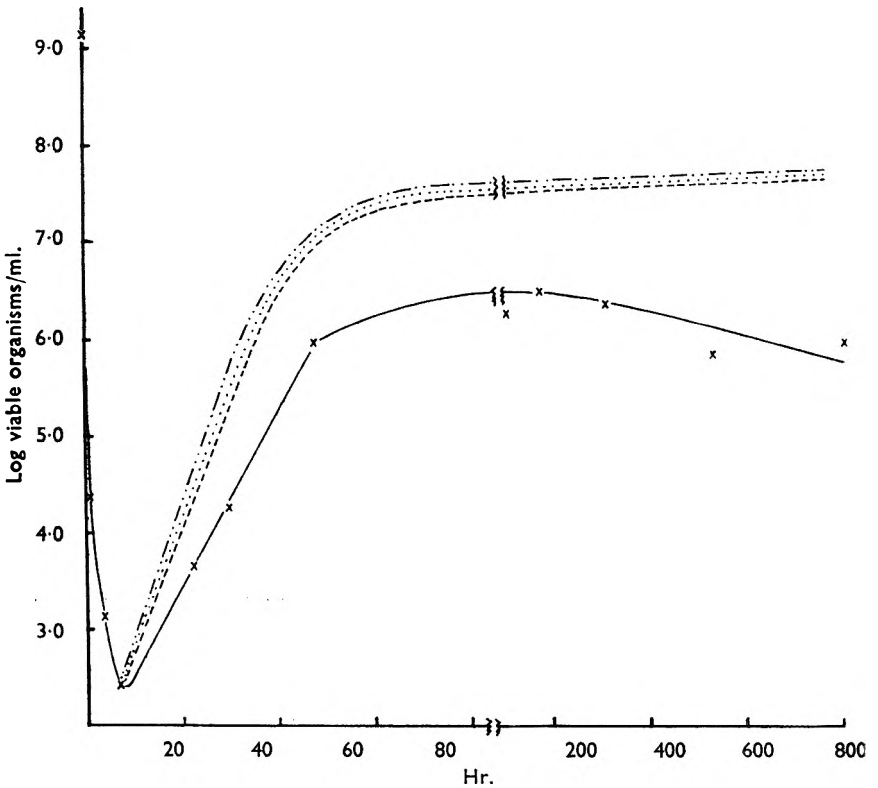

FIG. 3. Time-log survivor curves for *E. coli* in 50 μg./ml. benzylchlorophenol (×——×) and for organisms washed after 7½ hr. and re-suspended in solutions of cell exudate (broken lines).

–·–·–·– $d_{1\,cm.}$ at 260 mμ = 0·46
····· " " = 0·20
or – – – – – – distilled water.

in the earlier experiments, the viability of *E. coli* was determined in unbuffered solution of the phenol at 50 μg./ml.

Viable counts were made at intervals on *E. coli* in solutions containing respectively 20, 25, 27·5, 33·3, 50 and 100 μg./ml. of the phenol. The results, with the exception of those for the 100 μg./ml. solution, in which all the organisms died within 5 min., are presented in Figs. 2 and 3. In Fig. 2, the log survivor-time curves of *E. coli* in distilled water and in cell exudate solution ($d_{1\,cm.}$ = 1·25 at 260 mμ) are also shown for comparison.

The shape of the log survivor-time curve varied markedly with the concentration of the phenol. In the weakest solutions of 20 and 25 μg./ml., there was a gradual and continual decrease in the viable count which produced shallow sigmoidal curves. In the 27·5 μg./ml. solution, a rapid initial decrease in the viable count was followed by an equally rapid increase, and in the 33·3 and 50 μg./ml. solutions an extremely rapid decrease was followed by an almost equally rapid increase.

These results show conclusively that multiplication of the last survivors in solutions of benzylchlorophenol does occur in the absence of phosphate buffer and in concentrations of the phenol other than 50 μg./ml. A comparable multiplication of the last survivors in a solution of a chemical bactericide has not, to the authors' knowledge, hitherto been recorded, although Jacobs (1960) has recently observed multiplication of the last survivors of a suspension of *E. coli* in dilute broth containing phenol.

The viable counts during the period 250–1,100 hr. in all solutions containing benzylchlorophenol were within similar limits, indicating that common factors were limiting and controlling the multiplication of the last survivors. These factors are likely to be the same as those which produce the stationary phase of growth in liquid cultures, namely exhaustion of food supply, accumulation of toxic products or oxygen starvation.

Influence of Components of Reaction Mixture on the Viability of Last survivors

To justify the conclusion that the multiplication of the last survivors depended on the presence of cell exudate *E. coli* were treated with benzylchlorophenol, washed, and re-suspended either in sterile distilled water, cell exudate or solutions of the phenol, or in mixtures of the two latter.

Benzylchlorophenol solution 50 μg./ml. was selected as the bactericide because previous experiments had indicated that the survivors after 7·5 hr. could be as few as 1 in 10^7; the system would then contain an appreciable quantity of cell exudate. After 7·5 hr. 50 ml. quantities of the reaction mixture were centrifuged at 8,500 *g* for 3 min., the supernatant fluid removed, the organisms washed and re-suspended in the appropriate sterile fluid to produce approximately the same number of viable organisms as in the reaction mixture.

Viability after re-suspension in water and in exudate solutions. Two cell exudate solutions were used. One ($d_{1\ cm.}$ at 260 mμ = 0·20) was approximately equivalent in concentration to that produced by *E. coli* during 7·5 hr. in 50 μg./ml. of benzylchlorophenol and the other more than twice that concentration of exudate. After 7·5 hr. the reaction mixture contained only 252 viable cells/ml. (i.e. 2 in 10^7 of the original 2×10^9) survivors; the numbers in the reconstituted suspension in cell exudate solutions ($d_{1\ cm.}$ at 260 mμ = 0·46 and 0·20) and distilled water were 213, 179 and 218/ml. respectively. The viable count of the reconstituted suspensions increased more rapidly than that of the reaction mixture (Fig. 3). After 150 hr. about eleven times as many viable organisms were in the reconstituted suspensions as in the reaction mixture,

and at this time the count in the reaction mixture reached a peak indicating that the rate of multiplication in the reaction mixture was influenced by the residual concentration of the bactericide. At the end of the experimental period of nearly 800 hr. the reconstituted suspensions contained about 45 times as many viable organisms as did the control reaction mixtures. The rate of multiplication immediately after resuspension was greater in the cell exudate solutions than in the distilled water. Later, the differences between the rates diminished.

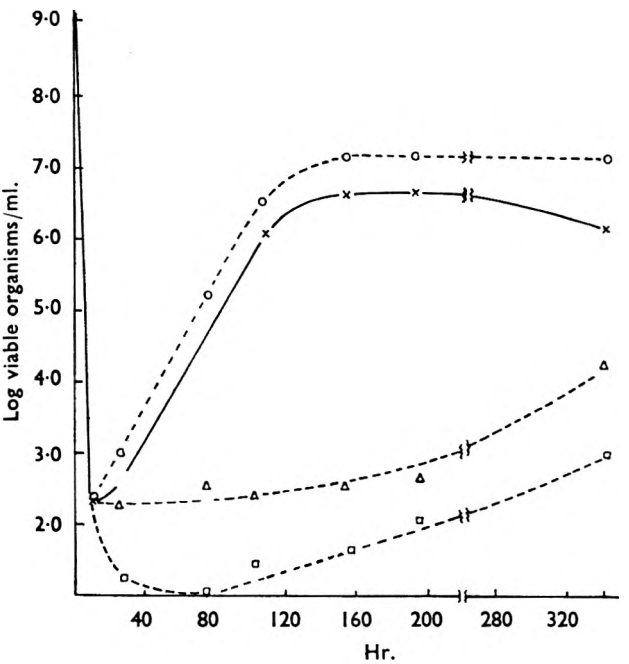

FIG. 4. Time-log survivor curves for *E. coli* in 50 μg./ml. benzylchlorophenol (×——×) and for organisms washed after 7½ hr. and re-suspended.

○——○ re-suspended in 20 μg./ml. benzylchlorophenol
△——△ " 30·75 " "
□——□ " 40 " "

Viability after re-suspension in solutions of benzylchlorophenol without added cell exudate. When *E. coli* had been suspended in benzylchlorophenol 50 mg./ml. for 7·5 hr., the residual bactericide in the supernatant was found to be 30·75 μg./ml. (Walters, 1959). To determine the influence of the phenol in the re-suspending medium, treated organisms were re-suspended in solutions containing 20, 30·75 and 40 μg./ml. of the phenol but no exudate. The increase in concentration retarded the increase in the viable count (Fig. 4). In the 20 μg./ml. re-suspension, multiplication was at a slightly greater rate than in the reaction mixtures. The 30·75 μg./ml. solution (i.e. the reaction mixture minus exudate), was bacteriostatic but after a considerable lag, multiplication occurred. The

40 μg./ml. solution was bactericidal. The survivors decreased to approximately 1 in 2×10^8 of the initial viable count of the reaction mixture, but ultimately multiplied as exudate became available from dying cells.

Viability after re-suspension in solutions of benzylchlorophenol with added cell exudate. The influence of different concentrations of cell exudate on multiplication was assessed by re-suspending treated organisms in benzylchlorophenol solution 30·75 μg./ml. without and with cell exudate

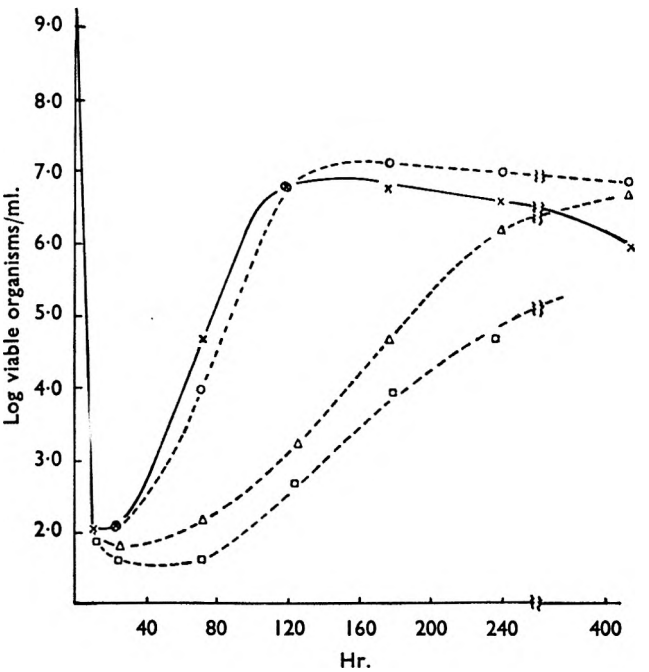

FIG. 5. Time-log survivor curves for *E. coli* in 50 μg./ml. benzylchlorophenol (×——×) and for organisms washed after 7½ hr. and re-suspended.

○ — — ○ re-suspended in 30·75 μg./ml. benzylchlorophenol + exudate ($d_{1cm.}$ at 260 mμ = 0·70)

△ — — △ re-suspended in 30·75 μg./ml. benzylchlorophenol + exudate ($d_{1cm.}$ at 260 mμ = 0·16)

□ — — □ re-suspended in 30·75 μg./ml. benzylchlorophenol. (No exudate added.)

added at the concentration estimated in the reaction mixture after 7·5 hr. ($d_{1\ cm.}$ at 260 mμ = 0·16) and at approximately 4½ times that concentration of exudate ($d_{1\ cm.}$ at 260 mμ = 0·70). When the re-suspension contained no cell exudate there was a further fall in the viable count, followed by a period of no-change in the count and ultimately by multiplication. The effect on the count of adding no exudate was, in fact, similar to that of increasing the supernatant concentration of the phenol in the reaction mixture, after 7·5 hr. (Figs. 4 and 5).

Re-suspending the treated organisms in a solution of the phenol and exudate at concentrations found in the control reaction mixture after

7·5 hr., caused a shorter lag than in the system containing no exudate (Fig. 5) but a longer lag and slower rate of multiplication than in the control. Increasing the exudate concentration 4·5-fold produced a growth cycle almost coincident with that of the control reaction mixture.

Re-suspension of E. coli *after Exposure to Benzylchlorophenol* 27·5 *and* 33·3 *μg./ml.*

Whether multiplication could be induced at different stages in the bactericidal reaction, was challenged with more dilute solutions containing 27·5 and 33·3 μg./ml. of benzylchlorophenol in which the reaction velocity

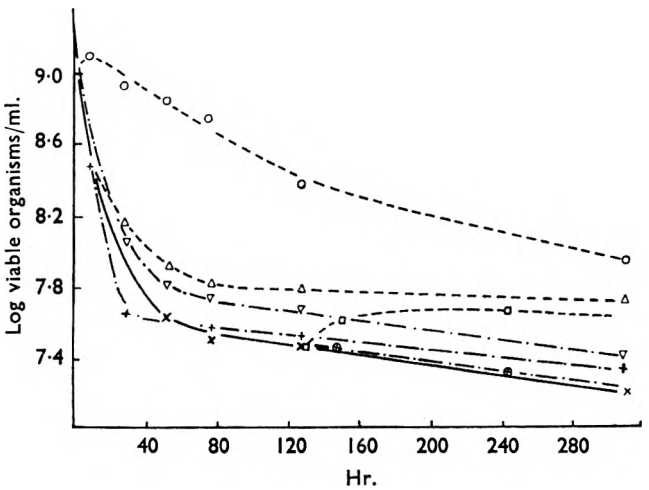

FIG. 6. Time-log survivor curves for *E. coli* in 27·5 μg./ml. benzylchorophenol (×——×) and for organisms washed after 1, 7 and 126 hr. and re-suspended.

○ — — ○ after 1 hr. re-suspended in cell exudate (d_{1cm} at 260 mμ = 0·15)
△ — — △ after 7 hr. re-suspended in cell exudate (d_{1cm} at 260 mμ = 0·17)
□ — — □ after 126 hr. re-suspended in cell exudate (d_{1cm} at 260 mμ = 0·38)
▽ — — ▽ after 1 hr. re-suspended in 20·5 μg./ml. benzylchlorophenol. No exudate.
+ — — + after 7 hr. re-suspended in 20·5 μg./ml. benzylchlorophenol. No exudate.
⊕ — — ⊕ after 126 hr. re-suspended in 20·5 μg./ml. benzylchlorophenol. No exudate.

was slower. The reactions were allowed to proceed until the survivors were estimated to approximate to 50, 20 and 0·1 per cent of the original population. Aliquots were therefore taken after the reaction had been in progress for 1, 7 and 126 hr. in the 27·5 μg./ml. solution and after 1 and 7 hr. in the 33·3 μg./ml. solution. The organisms were spun, washed and re-suspended, either in cell exudate or the phenol solution at the concentrations estimated in the supernatant fluid after the specified reaction periods. Multiplication was observed when the organisms were re-suspended in solution of cell exudate (Figs. 6 and 7). In both reaction mixtures the longer the duration of the reaction before re-suspension, the higher the mortality and the greater the rate of multiplication after re-suspension. This was expected since the higher mortalities produced greater amounts of cell exudate in the supernatant.

In the suspension of *E. coli* in solution of the phenol at 27·5 μg./ml. (Fig. 6) the mortality did not exceed 99·9 per cent and no multiplication was observed in the reaction mixture. It appeared that the resistance of the organisms had increased in the many months during which the experiments lasted since a mortality of 99·93 per cent followed by multiplication was previously observed with a solution of this concentration. The difference in behaviour of the organisms in the two experiments with 27·5 μg./ml. of the phenol emphasises that one factor determining whether or not multiplication occurs is the concentration of cell exudate in the supernatant fluid.

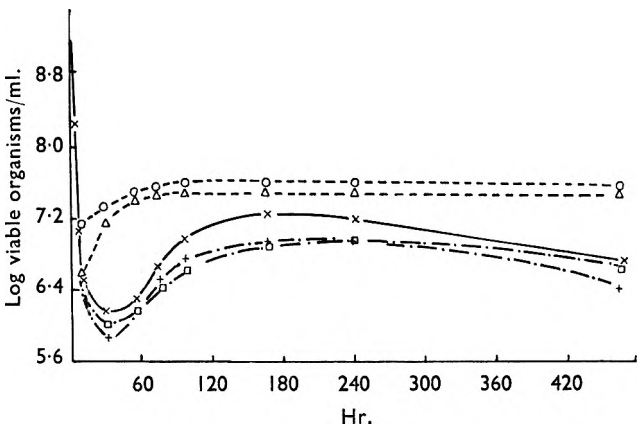

FIG. 7. Time-log survivor curves for *E. coli* in 33·3 μg./ml. benzylchlorophenol (×——×) and for organisms washed after 1 and 7 hr. and re-suspended.

○ — — ○ after 1 hr. re-suspended in cell exudate ($d_{1cm.}$ at 260 mμ = 0·16)
△ — — △ after 7 hr. re-suspended in cell exudate ($d_{1cm.}$ at 260 mμ = 0·18)
\+ — — + after 1 hr. re-suspended in benzylchlorophenol 25 μg./ml. No exudate
□ — — □ after 7 hr. re-suspended in benzylchlorophenol 25 μg./ml. No exudate

The time-survivor curves for the organisms re-suspended in solutions of benzylchlorophenol followed the same general pattern as the respective reaction mixture control curves. With the exception of the 1 and 7 hr. re-suspensions derived from the reaction mixture containing 27·5 μg./ml. of the phenol in which the mortality had not reached a low level, the viable counts for the re-suspensions were less than those for the control suspension. This indicated that in the normal reaction mixture the viable count, in particular at high mortality levels, was influenced and increased by the presence of cell exudate.

An attempt was made by direct microscopical count and by turbidity measurements to verify the increase in the viable count. The results were inconclusive. This is perhaps not surprising since an increase in the viable count from 2 to 3 × 10^2 per ml. to as many as 40 × 10^6 *E. coli* per ml. which were present in some of the cell exudate re-suspensions, represented a total increase of only 3 per cent of the initial viable count whereas the accuracy of microscopical counts is ±10 per cent (Wilson and Miles, 1955).

Discussion

The log time-survivor curves for the viability of *E. coli* in solutions of benzylchlorophenol varied from shallow sigmoidal curves to those showing an extremely rapid logarithmic decrease in the viable count followed by a rapid increase and finally a slow decline. The pattern of the increase in the count followed that represented by a typical bacterial growth curve and was similar to that of the multiplication of *E. coli* in cell exudate solution. It is concluded that the increase in the viable count was due to the multiplication of the last survivors and that the necessary nutrient was obtained from cell constituents released into the suspending fluid from dead and dying cells.

The following appear to be essential requirements for multiplication in a bactericidal system. Organisms which, upon their death, release cell constituents on which organisms of the same strain can multiply. A large inoculum to provide an adequate amount of cell exudate. A concentration of bactericide which reduces the viable population to a very low number ($>99{\cdot}9$ per cent mortality) without causing 100 per cent mortality, and thus producing a sufficient concentration of cell exudate for the multiplication of the survivors. A bactericide which is active in dilute solution and has a high concentration exponent so that removal of the bactericide from the solution by adsorption upon the bacterial surface considerably depletes the suspending fluid of bactericidal power.

The significance of the shape of the time-survivor curve is discussed at length in the literature. It is interpreted to indicate, on the one hand, that the bactericidal reaction is similar to a unimolecular chemical reaction and, on the other, that the length of time an organism can survive when exposed to a bactericide is proportional to its resistance to the bactericide. There are many instances of poor fit to the exponential equation. There are also many instances of small but significant numbers of survivors having been found after extended exposure times. Extrapolation of the time-survivor curves would in such cases have indicated very low probabilities of any survivors and sterility would have been assumed. This is nowhere more evident than in our own experiments and indicates that completely erroneous conclusions about sterilisation times may be drawn by the extrapolation of curtailed viable count observations. The evidence presented indicates that the application of the unimolecular law to the disinfection reaction should be regarded as no more than a convenient method of treating disinfection data. Clearly, it should be applied with extreme caution. This was emphasised by Chick (1910, 1930) and more recently, by other workers (Bullock, 1956; Hinshelwood, 1951; Phillips and Warshowsky, 1958).

It is possible that both the mechanistic theory of disinfection which assumes chance interactions of a bactericide with "sensitive" regions of cells possessing similar resistance (Chick, 1908, 1910; Rahn, 1945) and the vitalistic theory which assumes a progressive destruction of vital activities in cells possessing a variation in inherent resistance (Withell, 1942; Smith, 1921, 1923; Berry and Michaels, 1947b) together provide a truer explanation than does either alone for the maintenance

or loss of viability of organisms exposed to a bactericide, and hence for the shape of time-survivor curves. In the presence of a strong bactericide the speed of the chance events leading to death would dominate and mask any small difference in resistance between the cells. This would suggest apparently logarithmic survivor curves. Under mild disinfectant conditions any variation in the resistance of individual cells would be of more significance and the tendency would be towards sigmoidal survivor curves. The prolonged survival of cells towards the end of a mild disinfection process may also be due to increased resistance the cells acquire as a result of slow adaptive adjustment to the adverse conditions.

With the *E. coli*–benzylchlorophenol system a further explanation is necessary for the shape of the time-survivor curves. Multiplication of the small number of survivors remaining towards the end of the reaction occurs as a result of the utilisation of nutrient cell constituents released from dead cells.

Multiplication in an initially bactericidal system may be confined to reaction systems of this type. It is, however, possible that the reduction in the mortality rate with time which has been frequently observed towards the end of disinfection reactions, and which has been attributed to the greater resistance of the last survivors, may, in part, be due to the presence in the suspending fluid of cell constituents. Even if the last survivors do not multiply their survival times may be much prolonged. Certainly, neither the mechanistic nor the vitalistic theory of disinfection can alone offer a complete explanation for the course of the reaction reported in this communication.

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The paper was presented by DR. BEAN.

THE ASSAY OF ENZYME ACTIVITY BY THE PLATE-DIFFUSION TECHNIQUE

By D. V. Carter and G. Sykes

From the Microbiology Division, Standards Department, Boots Pure Drug Co. Ltd., Nottingham

The technique described provides a convenient means for estimating various enzymes quantitatively. Only a few examples are quoted, but provided suitable indicator substrates can be found the range can be extended. Having established the procedure for one enzyme, it is applicable to others, subject only to the variation in substrate required and the different dilutions of the enzyme preparation necessary to obtain satisfactory responses. Thus, the method is more convenient and easier to manipulate than are the existing methods, including those official in the B.P. and U.S.P.; moreover, as already stated, the assay is quantitative, the limits of error being only about 8 per cent ($P = 0.95$).

Enzymes will diffuse through an agar gel and digest a suitable substrate in the agar in the same way in which antibacterial substances diffuse to inhibit bacterial growth. Provided, therefore, that the extent of the digestion can be measured by some visible means, the principle of the cup-plate technique can be applied, and we have developed methods based on this principle for the assay of pepsin, trypsin and amylase but not lipase (in pancreatin), diastase and papain. The idea is not new; it was used, for example, by Dingle, Reid and Solomons (1953) mainly for determining saccharolytic enzyme activity during fungal metabolism. It also formed the basis of methods described by Castrèn (1956), but our methods differ from hers in several respects, notably in the enzyme concentrations needed to obtain satisfactory responses, and sometimes in the substrate used.

In contrast to the existing methods, the diffusion method allows a direct quantitative assessment of activity—the present methods are little more than qualitative, being simply "minimum performance" tests at given levels of dilution.

Experimental and results

The basis for the procedures described is the well-known four-point assay, that is, two dilution levels of the standard preparation are compared with two equivalent dilutions of the test material, the stronger dilution in both instances being four times that of the weaker. Large, flat-bottomed plates or trays measuring about 10 in. × 10 in. are used with an 8 × 8 Latin square design, thus allowing three samples to be examined simultaneously.

The diffusion medium in the plates is a gel of washed agar (1·2 per cent of Davis New Zealand agar has been found to be satisfactory) to which has been added a suitable concentration of a substrate appropriate for the enzyme being assayed. For storage purposes, the more concentrated gel from which this final dilution is made (see below) must be sterilised, but asepsis in the assay is not necessary.

The depth of agar-substrate mixture in the plates is about 1/10 in. (2·5 mm.), 180 ml. of agar-substrate being required for each dish, and the cups, cut with a No. 4 cork borer, are about 7 mm. diameter. The same volume of solution must be filled in each cup and for this a platinum-tipped dropping pipette is recommended. After filling, the plates are incubated at 37° for 16–18 hr.—neither the temperature nor the time of incubation is critical—and the diameters of the zones of digestion read, preferably with an optical projection device giving a magnification of about 5 diameters.

Calculations of the regression of zone diameter on log concentration of the enzymes show a linear relation. As an example, data and the

TABLE I

ASSAY OF PEPSIN BY THE DIFFUSION METHOD: SUBSTRATE 0·25 PER CENT CASEIN AT PH 3·5: RELATION BETWEEN LOG DILUTION AND ZONE DIAMETER

Replicate zone diameters at dilutions of:							
1:200		1:600		1:1,800		1:5,400	
23·0	23·0	19·0	19·5	17·0	17·0	15·0	15·0
21·2	22·0	19·5	21·0	18·0	17·2	15·0	15·2
22·0	21·5	20·2	20·0	17·7	18·0	15·0	15·2
22·0	21·7	20·0	20·2	17·2	17·2	15·7	15·0
22·0	22·5	19·0	19·7	18·0	17·0	15·0	15·0
21·2	21·0	20·5	19·2	17·5	17·5	15·5	15·0
22·8	22·0	19·5	19·5	17·0	17·2	15·0	15·2
22·0	21·0	19·5	19·2	17·0	17·7	15·2	15·7

Analysis of Variance

Source of variance	Sum of squares	Degrees of freedom	Mean squares
Between concentrations	409·329	3	
Regression	409·286	1	409·286***
Deviation from regression	0·043	2	0·022
Within concentrations (residual) ..	14·291	60	0·238 N.S.
Total	423·620	63	

*** = highly significant (P = < 0·001) N.S. = not significant (P = 0·1)

analysis of variance for pepsin are given in Table I. The limits of error are about 8 per cent (P = 0·95).

Pepsin. Casein, gelatin, haemoglobin and egg-white were tried as substrates in the assay of pepsin and casein proved the most suitable. Although the optimum activity of pepsin is at about pH 1·6, the best and most clearly defined zones occur at pH 3·5, the value chosen for all subsequent assays; at pH 5, the level used by Castrèn, the zones are much smaller and badly defined.

At pH 3·5, the casein is present as a suspension in the agar medium and the plates need to be prepared carefully. The method is:

To 90 ml. of sterile 2·4 per cent agar gel in water, previously melted and cooled not below about 75°, add 45 ml. of a 1 per cent solution of light soluble casein and then 45 ml. of double strength McIlvaine buffer at pH 3·5. By this means the casein is obtained as a finely dispersed suspension in the agar base. Pour immediately on a levelled plate and

allow to cool and solidify. Cut out the cups and fill them with the standard and test preparations diluted to the required concentrations with half-strength McIlvaine buffer at pH 3·5. The standard preparation should be prepared from pure pepsin, and suitable dilutions are given in Table II. (A 1 per cent solution of pepsin in acidified chloroform water is a convenient stock supply.)

Trypsin. The procedure is the same as for pepsin, except that a 4 per cent casein solution is used and the assay is carried out at pH 6, using McIlvaine buffer at this value. The zones of digestion show themselves as white haloes with a sharp outer edge on a clear background.

TABLE II

ASSAY OF ENZYMES BY THE DIFFUSION METHOD: RECOMMENDED DILUTIONS AND SUBSTRATES

Enzyme	Substrate and pH value	Recommended dilutions for:	
		Pure enzyme	Commercial preparations
Pepsin	Casein pH 3·5	1 : 1,600 and 1 : 6,400	B.P. "2,500" 1 : 50 and 1 : 200
			"10,000" 1 : 200 and 1 : 800
Trypsin	Casein pH 6	1 : 10,000 and 1 : 40,000	Pancreatin 1 : 200 and 1 : 800
Amylase	Starch pH 6	1 : 2,000 and 1 : 8,000	Pancreatin 1 : 200 and 1 : 800
Lipase		No reliable assay	
Diastase	Starch pH 6	Not available	B.P.C. '34 1 : 200 and 1 : 800
Papain	Casein pH 6	Not available	1 : 100 and 1 : 400

Using this method at the most suitable dilution levels (see Table II) the pure trypsin content of pancreatin was found to be between 1 and 2 per cent. An anomaly arose, however, in attempting to confirm this by the B.P. assay for trypsin. The pancreatin gave the expected results (an amino-acid titration equivalent to at least 3 ml of 0·1N NaOH) but trypsin, even with ten times the theoretical equivalent, gave only very low amino-acid titrations (0·5–1·0 ml.). When pure trypsin was added to pancreatin in sufficient quantity to double the activity in the diffusion assay, the B.P. method gave increases of only 25–50 per cent, and reducing the pH value and temperature of the B.P. assay did not effect these results. This indicated the presence of some controlling factor other than the trypsin.

Amylase. Amylase is most easily determined with starch as the substrate in an agar medium at pH 6. To prepare the plates, add 45 ml. of a 2 per cent starch gel in water, 45 ml. of McIlvaine buffer at pH 6 and about 1·8 g. sodium chloride (this is necessary as an activator for the enzyme) to 90 ml. of a 2·4 per cent agar gel in water, previously melted and cooled not below 75°, mix and pour on a levelled plate. After incubation, the zones of digestion appear only faintly, but they are

developed by flooding the plate with a 0·1 per cent solution of iodine in water, when they stand out as colourless zones on a blue background.

The recommended dilutions for the assay of pure α-amylase and amylase in pancreatin are given in Table II. It is essential that the amylase used as the standard be derived from pancreatic extracts since we have found that other α-amylases give different slopes in the assay.

Trypsin and amylase in pancreatin. Trypsin and amylase can easily be assayed simultaneously on one plate by combining the two methods just described. To prepare the plates, melt and cool not below 75° 90 ml. of agar gel as before and add to it 22 ml. of an 8 per cent solution of light soluble casein, 22 ml. of a 4 per cent starch gel in water, 45 ml. of double-strength McIlvaine buffer at pH 6 and about 1·8 g. sodium chloride, and pour on a levelled plate. After incubating, measure the diameters of the outer edges of the white haloes to estimate the trypsin content then flood the plate with 0·1 per cent iodine solution and measure the clear zone diameters on the blue background to estimate the amylase content.

Lipase. For the detection and estimation of lipase activity several substrates have been suggested: Castrén (1957) used Tween 20, Dingle, Reid and Solomons (1953) used polyethylene glycol monolaurate and Jones and Richards (1952) olive oil, butter fat or triacetin, each with a suitable indicator or developing agent. We have tried several of these, and other substances, but so far only triacetin, with bromocresol purple as the indicator, has shown any promise. The zones appear rapidly in 2–4 hr. but they are not clearly defined and more work in this direction is needed.

Diastase. The procedure for the assay of diastase is the same as that described for amylase and the definition of the zones is equally clear. The recommended concentrations with commercial diastase (Diastase B.P.C. '34) are given in Table II.

Papain. The same assay procedure is followed for papain as for trypsin, again with an optimum zone definition at pH 6. Above this value the zones become progressively less well defined and the characteristic halo disappears. Suitable concentrations of commercial papain to use in the assay are given in Table II.

Discussion

Although 37° is not necessarily the optimum for several of the enzymes examined, this temperature has been found most suitable in the diffusion assays described. At the higher temperature of 55°, for example, as specified in the B.P. assays for pepsin and trypsin, and even with shorter incubation periods, the zones are less regular, their edges are less well defined and the agar is liable to dry out during incubation; moreover it is generally more convenient to choose one temperature for all such assays rather than to have a different one for each enzyme. It is also desirable to limit the number of substrates, and for proteolytic enzymes casein has proved most suitable giving clear zones or haloes under the correct conditions and being readily available as a reasonably pure

substrate. Gelatin is probably the next most satisfactory substrate; it is more sensitive than casein, but is more variable in composition and involves a development stage in the assay with sulphosalicylic acid (Dingle and others, 1953) with the resulting zones less clearly defined. Egg-white, the substrate used in the B.P. assay for pepsin, is too variable and insufficiently sensitive in its response and haemoglobin, although giving reasonable zones, is not easy to use.

Where possible, comparative assays were made using the B.P. and the diffusion methods. Thus, taking into account the much greater precision of the latter, we have confirmed that Pepsin B.P. has only about one-twentieth of the enzymic activity of pure pepsin, and that pancreatin contains about 12 per cent of α-amylase. With trypsin, however, a similar comparison was not possible in that pure trypsin by the B.P. test appears to be almost inactive. This is because trypsin alone does not release the necessary amino-acids from casein as does trypsin in pancreatin. From this and the experimental evidence quoted earlier it is evident that the B.P. test is not a specific assay for trypsin but a composite one involving other enzymes which can further break down the tryptic degradation products to the amino-acids on which the B.P. test depends.

In the trypsin assay the white halos produced are of particular interest in that they only occur if the initial pH value is at 6·0. Even if the pH value is raised to only 6·5 no halos are produced and the digestion zones must be developed with trichloroacetic acid; similarly at pH 5·5 the casein is beginning to be precipitated and this interferes with the zone definition. At pH 6·0 the casein and its final digestion product is in solution, hence the insoluble material forming the halo appears to be an intermediate product.

In all assays by the diffusion method it is desirable to use the pure enzyme as the standard. Such material may not always be available, however, and for routine work standardised samples of commercial products can equally well be used.

Acknowledgments. The statistical calculations involved in this paper were made by Mr. R. Smart, to whom we wish to record our thanks. We would also thank Mr. G. Maxfield for his enthusiastic work at the bench.

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The paper was presented by Mr. Carter.

THE INFLUENCE OF THE VARIATIONS IN SOLUBILISING PROPERTIES OF POLYSORBATE 80 ON THE VITAMIN A PALMITATE : POLYSORBATE 80 : GLYCEROL : WATER SYSTEM

By P. F. G. Boon, C. L. J. Coles and M. Tait

From Glaxo Laboratories Ltd., Greenford, Middlesex

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The vitamin A palmitate: Polysorbate 80: glycerol: water system has been studied with special reference to the production of one-phase, water miscible, transparent solutions. The preparation of such solutions has been shown to depend on the hydrophile-lipophile balance of Polysorbate 80; this can be controlled by a cloud-point titration method.

Freedman and Green (1947) described the production of water-miscible preparations of lipophilic substances, such as vitamin A palmitate, with polyoxyethylene sorbitan fatty acid esters. Minimum proportions of such surfactants are desirable because they are bitter and expensive. To this end the value of glycerol in similar formulations was demonstrated by Coles and Thomas (1952). The United States Pharmacopeia XVI includes a monograph on Water-miscible Vitamin A Solution, describing the product as a viscous liquid, which on dilution with ten volumes of water may be clear or opalescent. Preparations are usually required to be transparent, of low viscosity and readily dispersible in aqueous media. For other purposes, however, such as encapsulation, a more viscous preparation may be required.

The object of this work was to chart the physical properties of the four component system and initially a specific sample of Polysorbate 80 was used. Attempts to reproduce certain preparations with further supplies of Polysorbate 80 were unsuccessful and the work was extended to investigate the effect of using different batches of the surfactant. Watanabe, Kanzawa, Mima, Yamamoto and Shima (1955) have reported a relationship between the hydrophile-lipophile character of polyoxyethylene sorbitan mono-oleate and its solubilising efficiency for vitamin A palmitate. Variations in hydrophile-lipophile character were therefore suspected as being responsible for our difficulties.

Experimental

Preparation of Phase Diagram

Preparations were made by dissolving the vitamin ester* (6·5 per cent by weight, equivalent to 100,000 I.U. A per gram of product) in the appropriate amount of Polysorbate 80† (polyoxyethylene sorbitan mono-oleate), adding glycerol B.P. while stirring and then diluting with water

* Vitamin A palmitate (Glaxo Laboratories Ltd., potency $1{\cdot}54 \times 10^6$ I.U. per gram: glyceride—free.

† Polysorbate 80 U.S.P. Supplied by Honeywill Atlas Ltd., and known commercially as Tween 80.

to a known weight. Integral ratios of Polysorbate 80: vitamin A palmitate, from 1:1 to 14:1, were examined, with 10 per cent increments in glycerol content.

Determination of Hydrophile-Lipophile Character

Greenwald, Brown and Fineman (1956) proposed a convenient water-titration method for the determination of hydrophile-lipophile character.

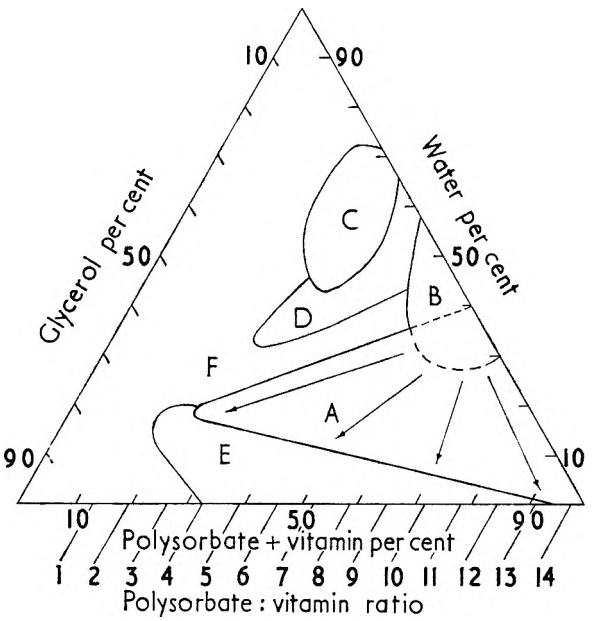

FIG. 1. The vitamin A: Polysorbate 80: glycerol: water system.
Variations in the character of preparations containing Polysorbate 80 (5 samples).

Zone	Description
A	Transparent, single phase
B	Semisolid
C	Faintly opalescent
D	Markedly opalescent
E	Two transparent phases
F	Emulsions.

A solution of the surfactant in a suitable solvent, here a 4 per cent v/v solution of benzene in dioxane, is titrated with water to a cloud-point. The titre (the "water-value") is a direct measure of the hydrophile-lipophile character of the surfactant and related to Griffin's (1949) hydrophile-lipophile balance value.

RESULTS

Assessment of the Phase Diagram

Fig. 1 represents the physical character of products that can be prepared with this system using one sample of the surfactant. Preparations are represented by points within the triangle. The percentages by weight of

glycerol and water are read from the left and right sides respectively. The percentage by weight of vitamin plus Polysorbate 80 is read from the base-line. Since the percentage of vitamin based on the final weight is constant at 6·5, the Polysorbate 80 content is conveniently expressed as a ratio of its weight to that of the vitamin ester. Zone A encloses all transparent single phase preparations. The ease of dilution with water depends on their viscosities, which, as indicated by the arrows, decrease radially from zone B, the region of semi-solid preparations. Faintly opalescent products of low viscosity occur in zone C; those in zone D

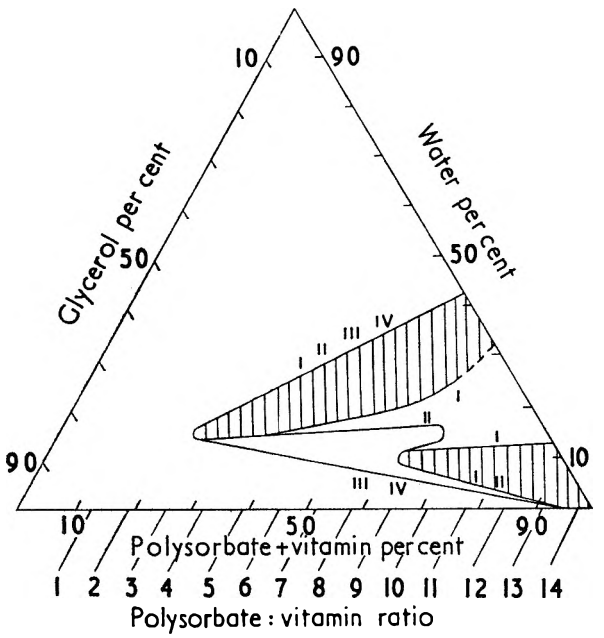

FIG. 2. Variations in the zone of transparent, single-phase preparations with different samples of Polysorbate 80.

Curve	Polysorbate 80 Sample	Water Value
I	2	10.3
II	4	12.3
III	5	12.7
IV	11	13.4

The shaded areas show where the samples of Polysorbate 80 all gave transparent, single-phase preparations.

are similar but have increased turbidity. Zone E encloses preparations that separate into two distinct phases. The remaining preparations (F) are opaque emulsions varying in physical stability. Separation of vitamin occurs in those of low-water content, and those of high-water content cream rapidly.

As indicated, a re-examination of the system with various samples of Polysorbate 80 revealed considerable variations in the area of zone A, which are illustrated in Fig. 2.

Hydrophile-Lipophile Character

Water values and polyoxyethylene content (Siggia, 1958) of several samples of Polysorbate 80 showed a rank correlation and are given in Table I.

Curves I to IV on Fig. 2 represent the results obtained with selected samples of Polysorbate 80 showing the effect of increasing water-values on the properties of the four component system.

TABLE I

WATER VALUES AND POLYOXYETHYLENE CONTENTS OF POLYSORBATE 80

Sample No.	Water value	Per cent (OCH_2CH_2)
1	10·0	74·2
2	10·3	76·0
3	12·2	
4	12·3	
5	12·7	76·9
6	12·8	
7	12·9	
8	13·1	77·3
9	13·2	
10	13·3	77·9
11	13·4	77·7

DISCUSSION

The best known quantitative approach to the concept of balanced surfactants is probably the hydrophile-lipophile balance (HLB) method of Griffin (1949). Originally the determination of HLB values was based on an assessment of emulsification performance, some seventy-five emulsions having been made for the determination of each HLB number. More recently Griffin (1954) devised formulae for calculating the HLB number from analytical data. The formulae relevant to the Polysorbate 80 type of surfactant are:

(1) $HLB = 20\,(1 + S/A)$
where S = saponification number of the ester, and A = acid number of the acid.

(2) $HLB = (E + P)/5$
where E = per cent oxyethylene, and P = per cent polyhydric alcohol.

The water-value determination is simpler in application, it has a much expanded scale between extreme samples and appears to be more related to the function of Polysorbate 80 in the formulations.

The areas of transparent, water-miscible preparations, given by all samples of Polysorbate 80 that were fully investigated, are indicated by shading in Fig. 2.

The considerable differences of area in Fig. 2 obtained from materials conforming to the U.S.P. XVI emphasise the need for a simple quantitative measure of solubilising power. Such a measure is provided by the water-value determination suggested by Greenwald and his colleagues.

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

The same batch of vitamin A palmitate was used throughout, its potency being from 1·5 to 1·6 $\times$ 10^6 I.U./g. It was not pure but the use of a single batch of impure material was still valid since the purpose of the paper was to demonstrate the variability in samples of polysorbate 80. The work was carried out in a constant temperature range between 24 and 26°.

PHASE EQUILIBRIA IN SYSTEMS CONTAINING COMMERCIAL NON-IONIC SURFACE-ACTIVE AGENTS AND BENZENE

BY B. A. MULLEY

From the School of Pharmacy, Chelsea College of Science and Technology, London, S.W.3

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IN recent years attempts have been made to describe solubilised systems by phase rule diagrams to establish the phenomena on a general basis. This approach should help to rationalise the often complicated solution behaviour of surface-active agents, and provides a firmer foundation for comparison between systems, since only solubility limits showing the same type of phase transitions should be compared when attempting to explain solubilities on a molecular or micellar basis.

Lawrence (1958) has published papers elucidating many features common to the various solubilised systems, giving the results in the form of ternary phase diagrams. Other workers have made isolated reports of similar phase diagrams. Most of this work was with anionic materials, usually at one temperature. A few cationic materials were also used. For many pharmaceutical purposes non-ionic surface-active agents are more useful, and have therefore been chosen for a comprehensive study of their solubilising properties. A preliminary account of part of this work has been given recently (Mulley, 1961), for some solutes and pure synthetic non-ionic agents of the alkyl polyether type (Mulley, 1958, 1960). The present paper gives uncompleted results obtained with three commercial surface-active agents, using benzene as the material to be solubilised.

The binary and ternary phase diagrams (Fig. 1) were obtained by visual examination of selected mixtures of the non-ionic surface-active agents with water and benzene, and by observing the effect of temperature on the phases present. Three commercial surface-active agents were used (Honeywill and Stein Ltd., London, W.1). They were Brij 30, Brij 35,

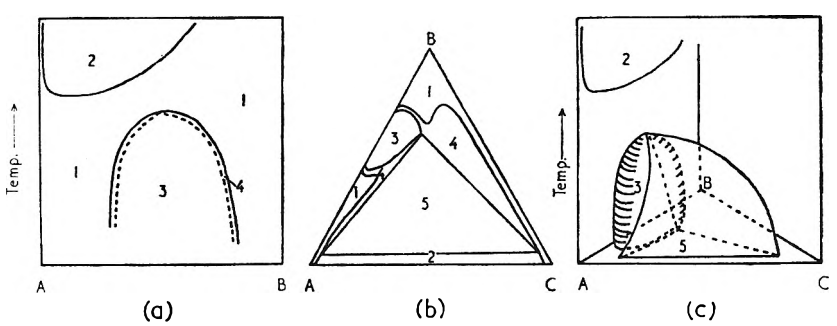

FIG. 1. Diagrammatic phase equilibrium drawings for binary and ternary systems containing non-ionic surface-active agents, benzene and water. (*a*) Binary diagram for the surface-active agents and water. (*b*) Ternary diagram at room temperature. (*c*) Ternary diagram showing the effect of temperature on regions 3 and 5. A, B and C = 100 per cent of water, surface-active agent and bezene respectively.

and Tween 60 and had the following (HLB) numbers respectively, 9·5, 16·9, and 14·9. The first two are polyoxyethylene lauryl ether derivatives with about 3·5 and 21 ethylene oxide units per molecule (calculated from the HLB values), and the other is a polyoxyethylene sorbitan monostearate. Temperatures between 5° and 100° were investigated in the binary systems, but only up to about 70° in the ternary systems owing to the lower boiling point when benzene is present.

In the binary mixtures the following regions and associated phases were found (excluding solid phases): region 1, a single isotropic liquid phase; region 2, two isotropic liquid phases; region 3, one anisotropic liquid crystalline phase; region 4, one isotropic liquid and one liquid crystalline phase. In the ternary systems containing benzene a fifth region is found where three phases are in equilibrium, two being isotropic liquids and the other liquid crystal. Diagrammatic representation of the relative positions of these regions is shown in Fig. 1. The phase boundaries are not accurately known in some regions. The liquid crystal phase existed up to the following temperatures in the binary systems, Brij 30, 72°; Brij 35, 56°; Tween 60, 77°. In the ternary systems it disappeared at lower temperatures in the Brij 30 and 35 mixtures, but continued above the boiling point in the Tween 60 system.

The pattern of phase behaviour found is remarkably similar to that described by Lawrence (1958) and agrees also with the few other diagrams in the literature. Although the concentrations and temperatures at which particular phases exist in different systems vary, the general arrangement of the regions for anionic, cationic and non-ionic surface-active agents and a number of different additives follows a common pattern, a fuller account of which has been given recently (Mulley, 1961).

The use of such phase diagrams in the formulation of solubilised products has already been pointed out by O'Malley, Pennati and Martin (1958). In addition, phase equilibria at very low concentrations of surface-active agents are important in the study of the mechanism of detergency (Lawrence, 1959; Mulley and Metcalf, *J. Colloid Sci.*, to be published). Moreover, changes in the nature of the phases present in emulsified systems, due to variation in temperature, are probably responsible, at least in part, for changes in their rheological properties and possibly for emulsion breakdown. The effect is marked with emulgents of the non-ionic type.

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The paper was presented by THE AUTHOR.

SOME OBSERVATIONS ON THE FLOW PROPERTIES OF BARIUM SULPHATE SUSPENSIONS

By J. E. Carless and N. C. Chenoy

From the Chelsea School of Pharmacy, Chelsea College of Science and Technology, London, S.W.3

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Barium sulphate particles of narrow size range were prepared by precipitation and the flow properties of suspensions of these particles were investigated. Particle sizes were determined by a photo-extinction sedimentometer method. The mean size of the particles varied from 6 μ to less than 1 μ. The flow properties of the suspensions can be explained by relating the viscosity not only to the volume concentration of the solid present but also to the volume of "free liquid" in the suspension. The Roscoe equation equating the relative viscosity to the concentration of a suspension, fits the experimental results, if an "effective concentration" is substituted for the actual concentration of solid. The "effective concentration" is the total volume of solid together with the solvent trapped within clumps of particles. This effective concentration was estimated from the sedimentation volume of the suspension.

The viscosity of a suspension is raised to a value η which is higher than the viscosity η_0 of the suspending liquid alone. The ratio η/η_0 is known as the relative viscosity (η_r) of the suspension. The basic equation of Einstein states that the relative viscosity of a suspension is related to the concentration of the solid present expressed as a fraction of the total volume (c):

$$\eta_r = 1 + 2{\cdot}5\,c$$

or $\eta_{sp} = 2{\cdot}5\,c$, where η_{sp} is the specific viscosity of the suspension.

This only applies to a dilute suspension of spherical monodispersed particles. Modifications of the equation to extend its application to higher concentrations have been proposed by numerous investigators. This aspect has been reviewed by Ward (1955). In most of the reported work on viscosity-concentration relations of suspensions, the particles have been relatively large, e.g., 10–100 μ. The present paper reports a preliminary investigation of the rheological properties of barium sulphate particles below 6 μ.

Experimental

Materials

The method of Andreason (1943) was used to prepare spherical, monodispersed particles of barium sulphate. The precipitated barium sulphate was washed with deionised water, then with ethanol (95 per cent), acetone and finally dried. Suspensions of varying volume concentrations were made up using a suspending medium of 40 per cent v/v glycerol containing 0·1 per cent w/v sodium pyrophosphate.

Methods

A Ferranti-Shirley cone plate viscometer as described by McKennell (1954) was used to measure the flow properties. All the measurements were made at 28° ± 0·1°.

The particle size analyses of the powders were made using a photo-extinction sedimentometer (Evans Electroselenium Ltd.) based on a design of Rose (1958). The technique has been described by Carless and Chenoy (1961).

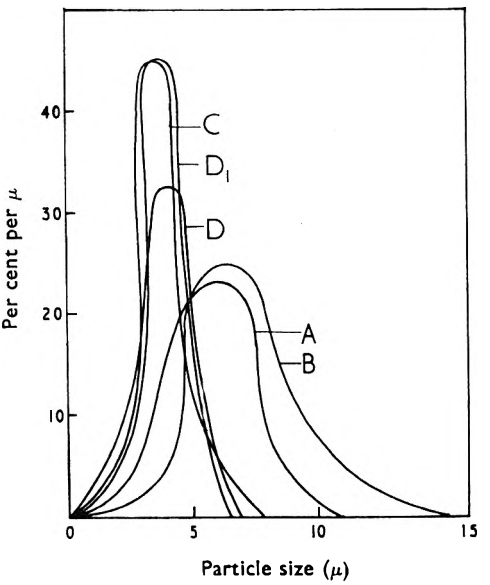

FIG. 1. Particle size distribution curves of barium sulphate powders.

The sedimentation volumes under gravity settling were determined by allowing 25 ml. of a 10 per cent v/v suspension of the sample in 0·1 per cent sodium pyrophosphate in water, to settle in a standard measuring cylinder for 7 days. The sedimentation volumes under centrifugal

TABLE I

PHYSICAL CHARACTERISTICS OF PRECIPITATED BARIUM SULPHATE POWDERS

Sample	Mean particle size (μ)	Shape	State of dispersion (5 per cent v/v suspension)
A	6·0	Cubical-spherical	Deflocculated
B	6·5	Spherical	Flocculated
C	3·75	"	Deflocculated
D	4·0	"	"
D_1	4·0	Irregular	"
E	<0·5	—	"

settling were determined by subjecting 10 ml. of a 10 per cent v/v suspension of the sample in the suspending medium, to a force of approximately 400 *g* for 10 min.

RESULTS AND DISCUSSION

The physical characteristics of the precipitated barium sulphate samples is shown in Table I, and the size distribution curves appear in Fig. 1.

Microscopic examination of the suspensions (5 per cent v/v) was made to check that the particles were effectively deflocculated. Of all the samples, only B was observed to be flocculated.

The variation of relative viscosity (η_r) with concentration (c) is shown in Fig. 2. All concentrations are expressed as v/v. Suspensions of A, B, C, D and D_1 were Newtonian at all concentrations used (1–25 per cent v/v). The suspension of E was Newtonian only up to 15 per cent v/v and at greater concentrations it showed thixotropy (see Fig. 3). The thixotropy is attributed to the sample being flocculated at high concentrations

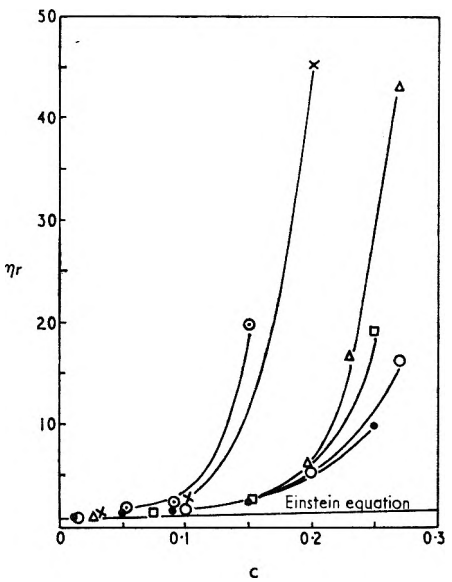

FIG. 2. Variation of relative viscosity with volume concentration.
● Sample A. △ Sample B. ○ Sample C. □ Sample D. X Sample D_1.
⊙ Sample E.

which results in a gel-like structure. It is interesting to note that this anomalous flow occurred only with the finest sample of powder. Similar behaviour for glass spheres in suspension has been reported by Williams (1953). The flow behaviour of suspensions of all samples above 10 per cent concentration showed a marked divergence from the Einstein equation. The results do not fit the equations postulated by Vand (1948) to account for hydrodynamic factors or by Roscoe (1952) to account for non-uniformity of the particles, agreement becoming less with decreasing particle size. Robinson (1949) postulated that the specific viscosity (η_{sp}) is not only a function of the volume concentration of the solid but is also a function of "free" liquid outside the particles or aggregates of particles. He suggested the following equation was applicable to a suspension of any concentration:

$$\eta_{sp} = \frac{Kc}{(1\text{-}Sc)}$$

Where c is the volume concentration and S the volume that a unit volume of particles would occupy when allowed to settle from a suspension. Thus 1-Sc is the volume of the free liquid. The plot of c/η_{sp} against c

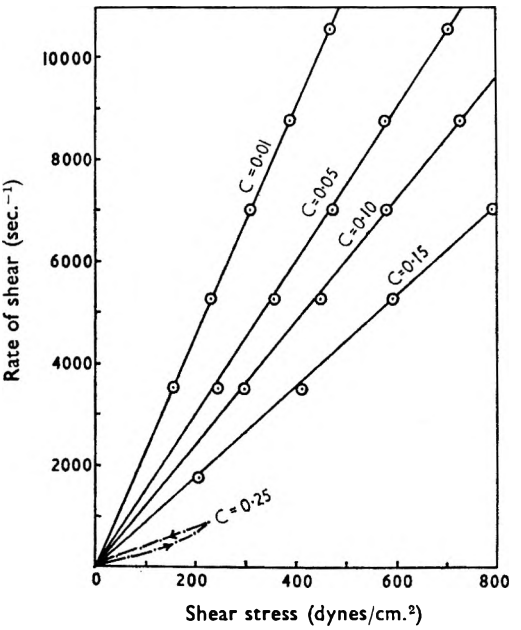

FIG. 3. Rate of shear *vs.* shear stress diagram for suspension of powder E.

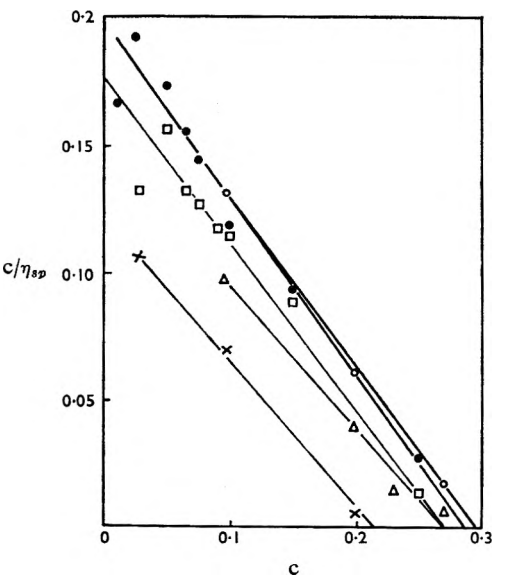

FIG. 4. Plot of c/η_{sp} *vs.* c.
● Sample A. △ Sample B. ○ Sample C. □ Sample D. X Sample D_1.

should yield a straight line with the intercept on the c axis being equal to $1/S$. The results for A, B, C, D and D_1 are shown in Fig. 4, and the predicted sedimentation volumes (S) appear in Table II together with the observed sedimentation volumes. The results show that the predicted values of S are similar to those values obtained from sedimentation volume measurement. This agreement appears to hold for both flocculated and deflocculated suspensions.

For uniform spheres in closest packing the voidage is 26 per cent, thus the theoretical sedimentation volume per unit volume of spheres, under ideal conditions, would be 1·35. From these observations it appeared likely that the reason for the variation between η_r *vs.* c curves (Fig. 2) for different powders might be caused by variation in the "effective con-

TABLE II

SEDIMENTATION VOLUMES OF BARIUM SULPHATE SUSPENSIONS

Sample	Sedimentation volume per unit volume of solid		
	Gravity*	Centrifuged*	S from viscosity measurements
A	2·3	2·4	3·6
B	3·2	3·4	3·7
C	2·4	—	3·4
D	3·0	3·1	3·7
D_1	4·2	4·0	4·7

* Concentration of suspension 10 per cent v/v.

centration" of the dispersed phase. An estimate of the effective concentration was obtained by multiplying the actual solid concentration by the ratio of the observed sedimentation volume to the theoretical sedimentation volume. Effective concentration for samples A, B, C, D and D_1 together with the η_r values appear in Fig. 5. The line represents the Roscoe equation for uniform spheres.

$$\eta_r = (1-1{\cdot}35\,c)^{-2{\cdot}5}$$

It appears therefore that all these suspensions show the same viscosity-concentration relation provided that allowance is made for the effective volume of solid.

Possible reasons for the observed sedimentation volumes being several times greater than the theoretical are: (i) immobilisation of fluid between clumps of particles; (ii) presence of an "immobilised liquid shell" around each particle.

From Fig. 2 it appears likely that flocculation is responsible for the difference in viscosity between A and B since the particle sizes are approximately the same. The structure of the aggregates is such that Newtonian flow is observed over a wide range of shear rates and concentration. Fine particles tend to aggregate to a greater extent than coarse ones so that the results in Fig. 2 are not unexpected. It is generally accepted that flocculation of particles is likely to be promoted on increasing the concentration of a suspension due to increased particle-particle contacts. In this present work the fact that the c/η_{sp} *vs.* c curve is linear, indicates that the proportion of flocculated solid to deflocculated solid remains constant over a wide concentration range.

The alternative explanation that the increased viscosity with decreased particle size is caused by an "immobilised liquid shell" would be reasonable if one assumes that the thickness of the shell is approximately the same for all particles, irrespective of size. Thus the effective volume increase would be greater for smaller particles and irregularly shaped particles. An estimate based on the observed sedimentation volumes,

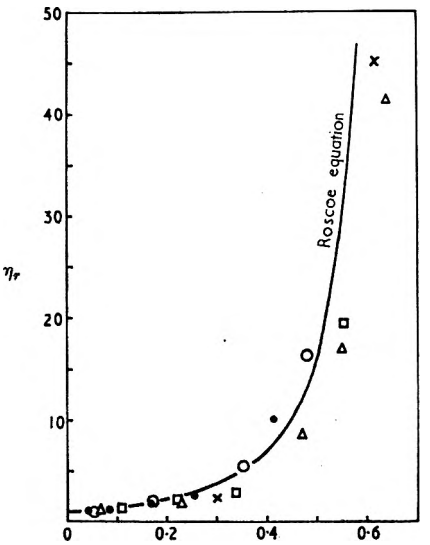

FIG. 5. Relation between relative viscosity and effective volume concentration of barium sulphate suspensions. (The line represents the Roscoe equation for uniform spheres (Roscoe, 1952).)

● Sample A. △ Sample B. ○ Sample C. □ Sample D. X Sample D_1.

indicates that the shell thickness would have to be about 1 μ. The Newtonian flow characteristics of the suspensions would support this explanation. However, this concept cannot explain the differences between deflocculated A and flocculated B, whose individual particles are of similar size. It appears more likely that trapping of the suspending medium between clumps of barium sulphate particles increases the concentration of the dispersed phase and this is responsible for the effects observed.

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The paper was presented by DR. CARLESS.

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SUBJECT INDEX*

A

* Page numbers followed by an italic *T* refer to the Supplement containing the Transactions of the British Pharmaceutical Conference.

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

INDEX OF AUTHORS*

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Z

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