JOURNAL OF PHARMACY
AND
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Successor to the Quarterly Journal of Pharmacy and Pharmacology

INCORPORATING THE YEAR-BOOK OF PHARMACY

VOLUME VIII, 1956

WITH THE

TRANSACTIONS

OF THE

BRITISH PHARMACEUTICAL

CONFERENCE

HELD AT

DUBLIN

SEPTEMBER 3 to 7, 1956

LONDON:
THE PHARMACEUTICAL PRESS
17, BLOOMSBURY SQUARE, W.C.1

Addendum

PERCUTANEOUS ABSORPTION USING DIIODOFLUORESCEIN ^{131}I

By J. W. Hadgraft, G. F. Somers and H. S. Williams

This Journal, 1956, **8**, 1027

Table I, *p.* 1030, column III, line 8. For — read 490.

Table II, *p.* 1030. Add footnote relating to Soft Paraffin and Lard reading: Averages of two experiments (i.e. 24 animals each).

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British Pharmaceutical Conference

NINETY-THIRD ANNUAL MEETING, DUBLIN, 1956

REPORT OF PROCEEDINGS

OFFICERS:

President:

GRANVILLE SHAW, F.P.S.

Chairman:

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Vice-Chairmen:

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E. F. HERSANT, B.Pharm., Ph.D., F.P.S., F.R.I.C., 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 CHAIRMAN and Honorary Secretary of the Local Committee (*ex officio*).

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*H. STEINMAN, M.P.S., Manchester.
T. D. WHITTET, B.Sc., F.P.S., F.R.I.C., London.

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

PROCEEDINGS OF CONFERENCE

DUBLIN, 1956

THE OPENING SESSION

The opening session of the Conference was held in the Ambassador Theatre, Dublin, on Monday, September 3, with Mr. Granville Shaw, President of the Conference (President of the Pharmaceutical Society) in the Chair. On the platform were the Chairman of the Conference (Prof. K. Bullock), the Lord Mayor of Dublin (Councillor R. T. Briscoe), the Chairman and Secretary of the Local Conference Committee (Mr. P. F. McGrath, President of the Pharmaceutical Society of Ireland, and Mr. D. J. Kennelly), the Honorary General Secretaries (Mr. H. G. Rolfe and Dr. E. F. Hersant), the Honorary Treasurer (Mr. H. Treves Brown), together with members of the Conference Executive Committee.

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

The President then handed over the further conduct of the Conference to the Chairman (Prof. K. Bullock), who delivered his address entitled "Biochemical Principles in Pharmacy," which is printed in full in the *Journal of Pharmacy and Pharmacology*, 1956, **8**, pages 689 to 708.

On the proposition of Dr. J. B. Stenlake, seconded by Mr. J. B. Lloyd, the Conference accorded a hearty vote of thanks to the Chairman for his address.

STATE RECEPTION

On the evening of Monday, September 3, a State Reception was held at the Gresham Hotel. The guests were received by the Minister for Local Government, Mr. P. O'Donnell and his sister-in-law, Mrs. R. J. Kelly and by the President of the Pharmaceutical Society of Ireland, Mr. P. McGrath and his daughter, Mrs. T. Butler. A dance was held at Clery's Ballroom after the reception.

THE SCIENCE SESSIONS

Meetings were held on Monday, Tuesday, Wednesday and Friday, September 3, 4, 5 and 7, at University College, the Chairman presiding. During the sessions the following 32 papers were communicated:—

1. Resistance of Crystalline Substances to Gas Sterilisation. By C. F. Abbott, B.Pharm., B.Sc., F.P.S., A.R.I.C., J. Cockton and W. Jones, F.P.S.
2. The Reproducibility of Extinction Time Estimates. Part III. Studies of Suspensions of Test Organisms. By A. M. Cook, B.Pharm., Ph.D., Dip.Bact., F.R.I.C., F.P.S., K. J. Steel, B.Pharm., F.P.S. and B. A. Wills, B.Pharm., Ph.D., M.P.S.
3. An Examination of Rubber Used as a Closure for Containers of Injectable Solutions. Part II. The Absorption of Chlorocresol. Part III. The Effect of the Chemical Composition of the Rubber Mix on Phenol and Chlorocresol Absorption. By W. T. Wing, F.P.S., D.B.A.
4. An Investigation into the Compaction of Powders. By D. Train, M.C., B.Pharm., B.Sc.(Eng.), Ph.D., A.C.G.I., D.I.C., F.P.S., A.R.I.C.
5. The Rheology of Oil-in-Water Emulsions I. The Effect of Concentration of Constituents on Emulsion Consistency. By A. Axon, M.S. (Wisconsin), B.Pharm., F.P.S.
6. Non-ionic Surface-active Agents. Part I. The Solubility of Chloroxylenol in Aqueous Solutions of Polyethylene Glycol 1000 Monocetyl Ether. By B. A. Mulley, B.Pharm., Ph.D., A.R.I.C. and A. D. Metcalf, B.Sc. (Pharm.).
7. The Chemical Assay of Cascara. By A. A. J. Fluck, B.Sc., W. Mitchell, B.Sc., Ph.D., F.R.I.C. and S. A. Wood, B.Sc.
8. A Source of Error in the Assay of Strychnine Salts and preparations Containing Strychnine. By A. C. Caws, B.Sc., A.R.I.C. and G. E. Foster, B.Sc., Ph.D., F.R.I.C.
9. Structural Requirements for Analgesic Activity in Alkyloxy-1-phenylethylamines and Some Views on Analgesic Mechanisms. By A. McCoubrey, B.Sc., Ph.D., M.P.S., F.R.I.C.
10. Analgesics and Their Antagonists: Some Steric and Chemical Considerations. Part I. The Dissociation Constants of Some Tertiary Amines and Synthetic Analgesics, and Their Implication in Considerations of the Conformations of Methadone-type Compounds. By A. H. Beckett, B.Sc., Ph.D., F.P.S., F.R.I.C.; Part II. The Influence of the Basic Group upon the Physico-chemical Properties and the Activity of Methadone and Thiambutene-type Compounds. By A. H. Beckett, B.Sc., Ph.D., F.P.S., F.R.I.C., A. F. Casy, B.Sc., F.P.S., A.R.I.C., N. J. Harper, M.Sc., Ph.D., Ph.C. (N.I.), A.R.I.C. and P. M. Phillips, B.Pharm. Part III. The Influence of the Basic Group on the Biological Response. By A. H. Beckett, B.Sc., Ph.D., F.P.S., F.R.I.C., A. F. Casy, B.Sc., F.P.S., A.R.I.C. and N. J. Harper, M.Sc., Ph.D., Ph.C. (N.I.), A.R.I.C.
11. The Determination of Riboflavine in Pharmaceutical Products. By L. Brealey, B.Sc. and D. A. Elvidge, B.Sc.
12. The Stability of B Vitamins in Pharmaceutical Products. By F. Wokes, B.Sc., Ph.D., F.P.S., F.R.I.C. and F. W. Norris, D.Sc., Ph.D., F.R.I.C., A.R.C.S., D.I.C.
13. The Determination of Benactyzine. By J. P. Jefferies, B.Sc., A.R.I.C. and J. I. Phillips, F.R.I.C.

14. *Glinus oppositifolius* L. Root—A Substitute for Senega. By R. M. Ridgway, B.Pharm., F.P.S. and J. M. Rowson, M.Sc., Ph.D., F.P.S., F.L.S.

15. The Evaluation of Belladonna Herb. Part I. The Quantitative Determination of Seed in Powdered Herb. By R. G. Atkinson, F.P.S., F.N.A.O., F.L.S. and C. Melville, B.Pharm., Ph.D., F.P.S.

16. The Pharmacognosy of Aspidosperma Barks of British Guiana. Part IV. Quantitative Numerical Studies of the Lignified Elements in Cascara and in *Aspidosperma* Species. By J. D. Kulkarni, B.Sc., Ph.D., B.Pharm., J. M. Rowson, M.Sc., Ph.D., F.P.S., F.L.S. and G. E. Trease, B.Pharm., F.P.S., F.R.I.C., F.L.S.

17. The Application of Enzyme Inhibition to the Estimation of Small Quantities of Drugs Possessing Anticholinesterase Activity. The Assay of Injection of Neostigmine Methylsulphate. By J. Buckles, B.Sc., F.P.S. and K. Bullock, M.Sc., Ph.D., F.P.S.

18. High Frequency Titrations in Pharmaceutical Analysis. By J. Allen, A.R.I.C., E. T. Geddes, and R. E. Stuckey, B.Sc., Ph.D., F.P.S., F.R.I.C.

19. A Method of Determining Binary Mixtures by Distribution Measurements, and its Application to the Assay of Strychnine in the Presence of Quinine. By C. Morton, B.Sc., F.P.S. and E. H. Tinley, B.Sc., F.P.S.

20. Sustained Release of Drugs from Ion Exchange Resins. By N. C. Chaudhry, B.Pharm. and L. Saunders, B.Sc., Ph.D., F.R.I.C.

21. The Sterilisation, Stability and Toxicity of Congo Red Injections. By G. F. Somers, B.Sc., Ph.D., F.P.S. and T. D. Whittet, B.Sc., F.P.S., F.R.I.C., D.B.A.

22. Percutaneous Absorption using Diiodofluorescein^{131}I. By J. W. Hadgraft, F.P.S., F.R.I.C., G. F. Somers, B.Sc., Ph.D., F.P.S. and H. S. Williams, B.Sc.

23. The Action of Ion Exchange Resins on Pyrogens. Part I. Effect on Pyrogenicity of Tap Water. By T. D. Whittet, B.Sc., F.P.S., F.R.I.C., D.B.A.

24. Paper Chromatographic Detection of New Constituents of *Digitalis lanata*. By Barbara J. Aldrich, M.P.S. (N.S.W.), Margaret L. Frith, M.P.S. (N.S.W.) and S. E. Wright, M.Sc., Ph.D., Dip.Pharm., A.R.I.C.

25. Studies in the Genus Digitalis. Part V. Fermentative Degradation of *D. purpurea* Leaf. By J. M. Rowson, M.Sc., Ph.D., F.P.S., F.L.S. and S. Simic.

26. Determination of the Digitoxin Content of *Digitalis purpurea*. By E. H. B. Sellwood, B.Pharm., F.P.S., A.R.I.C.

27. The Reactions of Antibacterial Substances with Bacteria. Methods using Nitrofurazone and *Aerobacter aerogenes*. By A. H. Beckett, B.Sc., Ph.D., F.P.S., F.R.I.C. and Ann E. Robinson, B.Pharm.

28. Antituberculosis Activity in the Phenazine Series. Isomeric Pigments obtained by Oxidation of *o*-Phenylenediamine Derivatives. By V. C. Barry, D.Sc., M. L. Conalty, M.D., D.P.H. and Ethna E. Gaffney, M.Sc., Ph.D.

29. The Chemistry of Santonin. Part II. Preparation of Some Derivatives with Possible Anthelmintic Activity. By W. Cocker, M.Sc., Ph.D., M.A., Sc.D. (Dublin), F.R.I.C., M.R.I.A. and T. B. H. McMurry, B.A.

30. Some Statistical Aspects of the Analytical Control and Standardisation of Tablets. By A. R. Rogers, B.Sc., B.Pharm, F.P.S., A.R.I.C.

31. Studies on *Leontice leontopetalum* Linn. Part I. Introduction. The Isolation of the Chemical Constituents of *Leontice leontopetalum* and Some Preliminary Observations on the Pharmacological Action of Leonticine and Petaline Chloride. By J. McShefferty, B.Sc., A.R.I.C., F.P.S., P. F. Nelson, B.Sc., M.P.S., J. L. Paterson, B.Sc., J. B. Stenlake, B.Sc., Ph.D., F.P.S., A.R.I.C. and J. P. Todd, Ph.D., F.P.S., F.R.I.C.

32. Studies on *Leontice leontopetalum* Linn. Part II. History, Sources and Macroscopical Characters of the Plant *L. Leontopetalum*. By P. F. Nelson, B.Sc., M.P.S. and F. Fish, B.Pharm, Ph.D., F.P.S.

The papers are printed in full with reports of discussions in the *Journal of Pharmacy and Pharmacology*, 1956, **8**, pages 709 to 804, 848 to 986 and 1019 to 1142.

THE SYMPOSIUM SESSION

A symposium on "Water" was held on Thursday, September 6. The CHAIRMAN presided. Two introductory papers were presented by Drs. E. W. Taylor and N. P. Burman, and E. Shotton and L. Saunders. The meeting is reported in the *Journal of Pharmacy and Pharmacology*, 1956, **8**, pages 817 to 847.

PROFESSIONAL SESSIONS

With the President of the Conference, Mr. Granville Shaw, in the chair, Professional Sessions were held on the mornings of Tuesday, September 4, when Mr. F. W. Adams read a paper on "Professional Conduct," and Friday, September 7, when Mr. H. W. Tomski read a paper on "Developments affecting Pharmaceutical Practice." Full reports of the papers and discussions were published in *The Pharmaceutical Journal*, 1956, **177**, 203–211, 235–242.

THE CLOSING SESSION

The closing session of the Conference was held on Friday, September 7, in the Great Hall of University College, Dublin, the Chairman, Prof. K. Bullock, presiding.

Vote of Thanks to the Dublin Committee

The Chairman called on Mr. P. G. Flood to propose a vote of thanks to the Local Committee. This was seconded by Mr. F. H. Oliver. The Chairman then presented to the Pharmaceutical Society of Ireland an ivory gavel provided from the Bell and Hills Fund. Mr. P. McGrath (Chairman of the Local Committee) replied to the vote of thanks and acknowledged the gift.

Annual Report

Mr. H. G. Rolfe presented the following Annual Report of the Executive Committee.

Your Executive have pleasure in presenting the ninety-third 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 66 members elected by the Executive.

Obituary.—The Executive report with deep regret the death, since the last Meeting, of F. W. Crossley-Holland, who was Honorary General Secretary, 1923 to 1927, Honorary Treasurer 1927 to 1934, and Chairman of the Conference in 1935.

Conference Research Papers.—Thirty-six papers were submitted and 32 accepted for presententation to the Conference. The Executive thank the authors for their contributions.

Journal of Pharmacy and Pharmacology.—The report of the meeting of the Conference at Aberdeen was published in the 7th Volume of the *Journal* of *Pharmacy and Pharmacology*. The Executive has been represented on the Editorial Committee by the Chairman (Professor K. Bullock), Professor J. P. Todd and the Senior Honorary General Secretary.

Relationship with the Society.—Discussions were concluded with the Council of the Society in regard to the future of their Branch Representatives' Meeting held during the Conference week. Your Executive is very gratified to be able to report that agreement was reached whereby the following changes have been made:—

(*a*) The Branch Representatives' Meeting has been discontinued as such and in its place, the Executive has agreed to arrange, as part of the Conference Programme, for the discussion of subjects of a professional nature on one whole day or two half days.

(*b*) The Society has agreed to send delegates from their branches to the Conference as a whole instead of to the Branch Representatives' Meeting only.

Future Meetings.—An invitation will be presented at this meeting for the Conference to meet in Bristol from September 2 to 6, 1957.

Several branches of the Society have made preliminary enquiries regarding the possibility of entertaining the Conference in future years and the Executive are grateful for all these offers of hospitality.

Constitution and Rules.—It is recommended that the Constitution and Rules be amended to include the Editor of the *Journal of Pharmacy and Pharmacology* as an additional *ex officio* member of the Executive Committee.

Officers and Executive of the Conference.—Your Executive have nominated the following Officers and members of the Executive for 1956—1957.

Chairman: Frank Hartley. *Vice-Chairmen:* R. R. Bennett, H. Deane, H. Humphreys Jones, T. E. Wallis, H. Brindle, B. A. Bull, Norman Evers, A. D. Powell, H. Berry, H. B. Mackie, G. R. Boyes, H. Davis, J. P. Todd and K. Bullock. *Honorary Treasurer:* H. Treves Brown. *Honorary General Secretaries:* H. G. Rolfe and E. F. Hersant. *Other members of the Executive:* K. R. Capper, J. W. Fairbairn, G. E. Foster, D. C. Garratt, J. B. Lloyd, W. Mitchell.

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

following *ex officio:* The Chairman of the Executive of the Scottish Department, the President of the Pharmaceutical Society of Ireland, the President of the Pharmaceutical Society of Northern Ireland, the Editor of the *Journal of Pharmacy and Pharmacology*, the Chairman of the Local Committee and the Honorary Local Secretary, will form the Executive for 1956–1957.

Mr. J. C. HANBURY proposed the acceptance of the report and the election of officers of the Conference for the ensuing year. Mr. W. T. WING seconded.

Mr. S. DURHAM, Sheffield, asked whether members of the Conference were entitled to make nominations for membership of the Executive.

Mr. H. G. ROLFE, in reply, read the relevant passage of the Constitution and Rules.

Dr. F. HARTLEY thanked the Conference on behalf of the newly-elected officers.

TREASURER'S REPORT

Mr. H. TREVES BROWN presented and proposed the adoption of the following Report and Statement of Accounts for the year 1956:—

During the year ended 31st December, 1955, the income from interest on investments, together with the donation of £25 from the Pharmaceutical Society of Northern Ireland and from the Pharmaceutical Society of Ireland, amounted to £86 16*s.* 10*d.* The sum of £41 6*s.* 3*d.* was spent in the purchase of six replicas of the Chairman's badge. Five of these are held in stock for future use (and are shown at cost as an asset in the Balance Sheet) and only the cost of the replica issued in 1955 has been charged in the Income and Expenditure Account. This, together with the purchase of a gavel as a memento to the Aberdeen Branch, made a total expenditure for the year of £14 13*s.* 9*d.*, leaving a surplus of £72 3*s.* 1*d.* to carry forward.

With the addition of this surplus the Accumulated Fund on 31st December, 1955, amounted to £1,871 3*s* 4*d.* In addition, the Balance Sheet contains the Local Committee Fund consisting of a donation of £250 from the London Committee 1953, held by the Executive to assist a Branch if necessary in the initial stages of preparation for a Conference. These two funds, amounting together to £2,121 3*s.* 4*d.*, are represented by: Cash, £50 7*s.* 10*d.*; Post Office Savings Bank Account, £586 8*s.* 0*d.*; Replicas of Chairman's badge £34 7*s.* 6*d.*; and 3% Savings Bonds, 1960–70, £200; together with the original donation by Alderman Clayton of Consols to the nominal value of £1,250. The realisable value of the Funds at the end of the year was £1,540. The large fall in the market value of the investments is not important as the investments are held for interest.

Subscriptions from elected members, including composition fee from the Pharmaceutical Society of Northern Ireland amounted to £134 10*s.* 0*d.* and were credited to the account of the *Journal of Pharmacy and Pharmacology*.

Expenditure of £928 1*s.* 6*d.*, which included £415 17*s.* 6*d.*, the cost of the scheme for assisting young pharmacists, paid by the Pharmaceutical Society of Great Britain towards the general expenses of the Conference, was included in the Pharmaceutical Society's Financial Statement presented at the Annual Meeting in May of this year.

BRITISH PHARMACEUTICAL CONFERENCE ACCOUNT

INCOME AND EXPENDITURE ACCOUNT, 1955

Expenditure	£	*s.*	*d.*	*Income*	£	*s.*	*d.*
Gavel—memento to Aberdeen Branch	7	15	0	Interest on 2½% Consols	22	12	10
Replicas of Chairman's Badge and engraving *less* stock carried forward	6	18	9	Interest on 3% Savings Bonds ..	6	0	0
Surplus carried to Balance Sheet ..	72	3	1	Interest on P.O. Savings Bank Account	8	4	0
				Donation from Pharmaceutical Society of Northern Ireland ..	25	0	0
				Donation from Pharmaceutical Society of Ireland	25	0	0
	£86	16	10		£86	16	10

BALANCE SHEET AT 31ST DECEMBER, 1955

Liabilities	£	*s.*	*d.*	*Assets*	£	*s.*	*d.*
Accumulated Fund, as at 31.12.54 ..	1,799	0	3	Investments (*a*) £1,250 2½% Consols (Donation by the late Alderman Clayton of Birmingham) (Market value at 31st December, 1955: £706)	1,250	0	0
Add Surplus 1955	72	3	1				
	1,871	3	4				
Local Committee Fund:—							
Donation from London Committee, 1953..	250	0	0	Investments (*b*) £200 3% Savings Bonds 1960–70 (Market value at 31st December, 1955: £163) ..	200	0	0
				Stock of Replicas of Chairman's Badge	34	7	6
				Post Office Savings Bank Account ..	586	8	0
				Cash at Westminster Bank	50	7	10
	£2,121	3	4		£2,121	3	4

H. TREVES BROWN,
Honorary Treasurer.

The President seconded, and the Report was adopted.

British Pharmaceutical Conference

INAUGURAL MEETING HELD AT NEWCASTLE-ON-TYNE IN 1863

Years	*Places of Meeting*	*Presidents*	*Local Secretaries*
1864	BATH	HENRY DEANE, F.L.S.	J. C. POOLEY.
1865	BIRMINGHAM ..	HENRY DEANE, F.L.S.	W. SOUTHALL, JUN.
1866	NOTTINGHAM ..	PROF. BENTLEY, F.L.S.	J. H. ATHERTON, F.C.S.
1867	DUNDEE ..	PROF. BENTLEY, F.L.S.	J. HODGE.
1868	NORWICH ..	DANIEL HANBURY, F.R.S.	F. SUTTON, F.C.S.
1869	EXETER ..	DANIEL HANBURY, F.R.S.	M. HUSBAND.
1870	LIVERPOOL ..	W. W. STODDART, F.C.S.	E. DAVIES, F.C.S. J. DUTTON (Birkenhead).
1871	EDINBURGH ..	W. W. STODDART, F.C.S.	J. MACKAY, F.C.S.
1872	BRIGHTON ..	H. B. BRADY, F.R.S.	T. GLAISYER.
1873	BRADFORD ..	H. B. BRADY, F.R.S.	R. PARKINSON, PH.D.
1874	LONDON ..	THOS. B. GROVES, F.C.S.	M. CARTEIGHE, F.C.S.
1875	BRISTOL ..	THOS. B. GROVES, F.C.S.	J. PITMAN.
1876	GLASGOW ..	PROF. REDWOOD, F.C.S.	A. KINNINMONT.
1877	PLYMOUTH ..	PROF. REDWOOD, F.C.S.	R. J. CLARK.
1878	DUBLIN ..	G. F. SCHACHT, F.C.S.	W. HAYES.
1879	SHEFFIELD ..	G. F. SCHACHT, F.C.S.	H. MALEHAM.
1880	SWANSEA ..	W. SOUTHALL, F.L.S.	J. HUGHES.
1881	YORK	R. REYNOLDS, F.C.S.	J. OWRAY.
1882	SOUTHAMPTON	PROF. ATTFIELD, F.R.S.	O. R. DAWSON.
1883	SOUTHPORT ..	PROF. ATTFIELD, F.R.S.	WM. ASHTON.
1884	HASTINGS ..	J. WILLIAMS, F.C.S.	F. ROSSITER.
1885	ABERDEEN ..	J. B. STEPHENSON.	A. STRACHAN.
1886	BIRMINGHAM ..	T. GREENISH, F.C.S.	CHAS. THOMPSON.
1887	MANCHESTER ..	S. R. ATKINS, J.P.	F. B. BENGER, F.C.S.
1888	BATH	F. B. BENGER, F.C.S.	H. HUTTON.
1889	NEWCASTLE-ON-TYNE	C. UMNEY, F.I.C., F.C.S.	T. M. CLAGUE.
1890	LEEDS	C. UMNEY, F.I.C., F.C.S.	F. W. BRANSON, F.C.S.
1891	CARDIFF ..	W. MARTINDALE, F.C.S.	ALFRED COLEMAN.
1892	EDINBURGH ..	E. C. C. STANFORD, F.C.S.	PETER BOA.
1893	NOTTINGHAM ..	OCTAVIUS 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.	STEWART HARDWICK.
1896	LIVERPOOL ..	W. MARTINDALE, F.C.S.	T. H. WARDLEWORTH. H. O. DUTTON (Birkenhead).
1897	GLASGOW ..	DR. C. SYMES.	J. A. RUSSELL.
1898	BELFAST ..	DR. C. SYMES.	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. HERBERT 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., F.C.S.	H. E. BOORNE.
1904	SHEFFIELD ..	T. H. W. IDRIS, M.P., F.C.S.	H. ANTCLIFFE.
1905	BRIGHTON ..	W. A. H. NAYLOR, F.I.C., F.C.S.	W. W. SAVAGE. C. G. YATES.
1906	BIRMINGHAM ..	W. A. H. NAYLOR, F.I.C., F.C.S.	C. THOMPSON.
1907	MANCHESTER ..	THOS. TYRER, F.I.C., F.C.S.	W. KIRKBY.
1908	ABERDEEN ..	ROBT. WRIGHT, F.C.S.	W. F. HAY.
1909	NEWCASTLE-ON-TYNE	J. G. TOCHER, B.Sc., F.R.I.C.	T. M. CLAGUE. H. W. NOBLE.
1910	CAMBRIDGE ..	FRANCIS RANSOM, F.C.S.	A. A. DECK. T. J. MALLETT.
1911	PORTSMOUTH ..	W. F. WELLS.	T. O. BARLOW. T. POSTLETHWAIT.
1912	EDINBURGH ..	SIR EDWARD EVANS, J.P.	THOS. STEPHENSON.
1913	LONDON ..	JOHN C. UMNEY, F.C.S.	W. J. UGLOW WOOLCOCK.
1914	CHESTER ..	E. H. FARR, F.C.S.	R. CECIL OWEN, B.Sc.
1915	LONDON ..	E. SAVILLE PECK, M.A.	———
1916	LONDON ..	DAVID HOOPER, LL.D., F.R.I.C.	———
1917	LONDON ..	CHAS. ALEX. HILL, B.Sc., F.R.I.C.	———
1918	LONDON ..	CHAS. ALEX. HILL, B.Sc., F.R.I.C.	———
1919	LONDON ..	W. KIRBY, M.Sc., F.C.S.	———
1920	LIVERPOOL ..	CHAS. ALEX. 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, Dès. 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	EDMUND WHITE, B.Sc., F.I.C.	P. J. THOMPSON. W. H. HALLETT.
1925	GLASGOW ..	EDMUND 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, M.B., B.S., B.Sc., F.R.I.C.	H. N. LINSTEAD.
1934	LEEDS	C. H. HAMPSHIRE, M.B., B.S., B.Sc., F.R.I.C.	G. C. CRUMMACK.
1935	BELFAST ..	F. W. CROSSLEY-HOLLAND, L.M.S.S.A.	D. L. KIRKPATRICK.
1936	BOURNEMOUTH	HAROLD DEANE, B.Sc., F.R.I.C.	V. J. SCAMPTON.
1937	LIVERPOOL ..	T. EDWARD 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. RUSHTON.
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 ..	NORMAN EVERS, PH.D., F.R.I.C.	A. WILSON.
1949	BLACKPOOL ..	NORMAN EVERS, PH.D., F.R.I.C.	P. VARLEY. T. A. DURKIN.
1950	GLASGOW ..	A. D. POWELL, F.R.I.C.	A. OFFICER.
1951	HARROGATE ..	H. BERRY, B.Sc., Dip. Bact. (London), 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.	G. L. DICKIE.
1956	DUBLIN ..	K. BULLOCK, M.Sc., PH.D., F.R.I.C.	D. J. KENNELLY.

Honorary Treasurers (One)

1863 to 1870, H. B. BRADY, F.R.S.
1870 to 1877, GEORGE F. SCHACHT, F.C.S.
1877 to 1884, C. EKIN, F.C.S.
1884 to 1888, C. UMNEY, F.I.C., F.C.S.
1888 to 1890, W. MARTINDALE, F.C.S.
1890 to 1893, R. H. DAVIES, F.I.C., F.C.S.
1893 to 1898, JOHN MOSS, F.I.C., F.C.S.
1898 to 1912, JOHN C. UMNEY, F.C.S.
1912 to 1925, D. LLOYD HOWARD, J.P., F.C.S.
1925 to 1927, R. R. BENNETT, B.Sc., F.R.I.C.
1927 to 1934, F. W. CROSSLEY-HOLLAND, L.M.S.S.A.
1934 to 1936, T. E. LESCHER, O.B.E.
1936 to 1940, A. R. MELHUISH.
1940 to 1947, T. MARNS.
1947 to 1952, G. R. BOYES, L.M.S.S.A., B.Sc., F.R.I.C.
1952 to 1953, H. DAVIS, B.Sc., PH.D., F.R.I.C.
1953 to H. TREVES BROWN, B.Sc.

Honorary General Secretaries (Two)

1863 to 1880, PROF. ATTFIELD, PH.D., F.R.S.
1863 to 1871, RICHARD REYNOLDS, F.C.S.
1871 to 1884, F. BENGER, F.C.S.
1880 to 1882, M. CARTEIGHE, F.C.S.
1881 to 1886, SIDNEY PLOWMAN, F.R.C.S.
1884 to 1890, JOHN C. THRESH, M.B., D.Sc.
1886 to 1901, W. A. H. NAYLOR, F.I.C., F.C.S.
1890 to 1903, F. RANSOM, F.C.S.
1903 to 1909, EDMUND WHITE, B.Sc., F.I.C.
1901 to 1921, E. SAVILLE PECK, M.A.
1909 to 1919, HORACE FINNEMORE, B.Sc., F.R.I.C.
1912 to 1923, R. R. BENNETT, B.Sc., F.R.I.C.
1919 to 1929, C. H. HAMPSHIRE, C.M.G., M.B., B.S., B.Sc., F.R.I.C.
1923 to 1927, F. W. CROSSLEY-HOLLAND, L.M.S.S.A.
1927 to 1944, C. E. CORFIELD, B.Sc., F.R.I.C.
1929 to 1947, G. R. BOYES, L.M.S.S.A., B.Sc., F.R.I.C.
1944 to 1953, H. TREVES BROWN, B.Sc.
1947 to H. G. ROLFE, B.Sc., F.R.I.C.
1953 to E. F. HERSANT, B.Pharm., PH.D., F.R.I.C.

Closing Session (continued).

PLACE OF MEETING FOR 1957

Mr. K. J. ADAMS (Chairman of the Bristol and District Branch) extended an invitation to hold the Conference in Bristol in 1957.

Mr. H. H. CAMPBELL proposed that the invitation be accepted, and Dr. J. C. PARKINSON seconded. The vote was put to the meeting and unanimously carried.

VOTE OF THANKS TO CHAIRMAN

Dr. E. SHOTTON proposed a vote of thanks to the Chairman.

Mr. C. E. TURNER seconded. The vote was put to the meeting by the President and carried with acclamation.

Prof. BULLOCK briefly responded.

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 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 35*s*. annually, which shall entitle them, on application, to receive the *Journal of Pharmacy and Pharmacology*, as published. Subscription shall become due on January 1, and membership shall cease if subscriptions are not paid by June 1.

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REVIEW ARTICLE

PATHWAYS OF DRUG METABOLISM*†

BY BERNARD B. BRODIE, B.Sc., Ph.D.

Chief Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland

THE action of a drug would probably last a lifetime if the body did not have ways of limiting its duration. In this lecture I shall discuss the nature of a number of these mechanisms. At one time the kidney was considered the most important organ in enabling the body to dispose of drugs. But it is becoming more and more evident that the kidney usually excretes only a small proportion of a drug in an unchanged form and the bulk as inactive derivatives. Of course there are notable exceptions: tolazoline, an adrenergic blocking agent, is excreted almost entirely unchanged; a number of sulphonamides are only in part metabolised; and a considerable fraction of penicillin is found unchanged in urine. But, by and large, the action of most drugs is terminated by their biotransformation. Although the biotransformation of a vast number of drugs in the whole organism has been studied, we have known very little, until recently, of the nature of enzymatic mechanisms concerned in these processes of "detoxication."

The fate of drugs in the body has interested our laboratory for a number of reasons. At one time we considered it possible that biotransformation mechanisms might explain how certain drugs exert their pharmacological action; that is, drugs in being metabolised might become enmeshed in mechanisms involved in the normal economy of the body and thus interfere with normal function. As we shall see later, this viewpoint is difficult to entertain since most drugs are not measurably metabolised in the organ where they act. Another reason for our interest in drug metabolism was the possibility that this might be a backdoor approach to general biochemistry, with the drug being used as a bait to induce unknown enzymes to disclose themselves. Finally, it is thought that a detailed knowledge of enzymes involved in drug "detoxication" might help the medicinal chemist to develop compounds of either high or low stability in the body, whichever would be more desirable in gaining a desired therapeutic result.

THE INHIBITORY ACTION OF SKF 525-A

Although certain aspects of this programme had been going on in our laboratories for some time, it was a phone call from Dr. Glenn Ullyot

* This article is based on the first of two University of London special university lectures in pharmacology on "Biochemical and Clinical Implications of Studies in Drug Metabolism," given at Guy's Hospital Medical School, May 17, 1954. A second article will appear later.

† The studies presented here include the work of Drs. J. Axelrod, J. Cooper, J. Fouts, J. Gillette, B. N. La Du, C. Mitoma, G. Quinn, and S. Udenfriend.

of Smith, Kline and French Laboratories in Philadelphia that really "sparked" our studies. He told us that their laboratory had developed a strange compound (SKF 525–A), a congener of Trasentin that lacked its spasmolytic activity. Thinking that it might have sedative action, they determined the effect of the compound in prolonging the duration of hypnosis induced by hexobarbitone and other barbiturates. For example, rats were given a dose of hexobarbitone which ordinarily resulted in their sleeping for about 40 minutes. However, when pretreated with a 100 mg. per kg. dose of SKF 525–A they now slept for several hundred minutes. Obviously, this was more than an additive effect. In fact, SKF 525–A by itself was subsequently shown to exhibit no sedative effect whatever, or any other obvious effect except at very large doses. We were also told that SKF 525–A prolonged the action of the narcotic analgesics—pethidine, codeine, and morphine—and of mephenesin, a centrally-acting muscle relaxant. But all the drugs thus far mentioned depressed the central nervous system and it seemed possible to us that SKF 525–A might affect some central component common to them all. But on hearing that

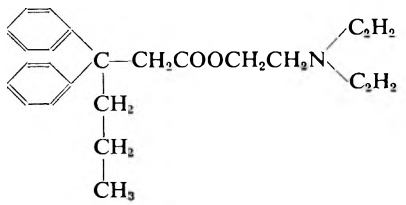


TABLE I

EFFECT OF SKF 525–A ON METABOLISM OF HEXOBARBITONE IN RATS

Rats received 100 mg./kg. hexobarbitone intraperitoneally (control). One week later experiments repeated except that 15 mg./kg. SKF 525–A was given before the hexobarbitone injection.

Rat No.	Duration of action*		Hexobarbitone half-life		Plasma level on awakening*	
	Control min.	SKF 525–A treated min.	Control min.	SKF 525–A treated min.	Control mg./l	SKF 525–A treated mg./l
1	21	89	24	69	48	38
2	27	90	24	60	42	34
3	34	86	32	71	40	41
4	23	68	23	49	27	33
5	28	68	27	50	46	38

* Return of righting reflex.

SKF 525–A also prolonged the action of (+)-amphetamine, a central nervous system stimulant[1–5], we realised this compound might be extremely useful in the study of drug action. We are grateful to Smith, Kline and French for giving us the opportunity of investigating their compound, for it has proved a particularly rewarding tool in providing a uniform picture of the biotransformation process of a variety of drugs.

We first studied the effect of SKF 525–A on barbiturate drugs *in vivo*[6]. The sleeping times of rats injected with the new compound and hexobarbitone were compared with those of rats injected with hexobarbitone alone. Table I shows that rats pretreated with 15 mg. per kg. of SKF 525–A slept three times as long as the same animals given barbiturate alone. The rate of metabolism of the barbiturate was affected, the

biological half-life (the time required for disappearance of half the drug from the body) being prolonged about threefold. With larger doses of SKF 525–A this action was more pronounced; for example, with 50 mg. per kg. the biological half-life of hexobarbitone increased about fivefold. Of particular interest was the finding that plasma concentrations of hexobarbitone when the rats awoke were roughly the same whether or not the animals had received SKF 525–A (Table I), indicating that the potentiator did not act by sensitising the site of action of the barbiturate. It may be concluded that the increased duration of action resulted only from inhibition of the metabolic transformation of the barbiturate. An inhibitory effect on the metabolism of hexobarbitone was also demonstrable in other animal species. As shown in Table II, SKF 525–A decreased the rate of biotransformation and increased the duration of action of the hexobarbitone in dogs and these effects were markedly enhanced with an increase in dosage of the former. The metabolism of other barbiturates such as pentobarbitone and quinalbarbitone was also depressed. Since barbiturates are metabolised mainly through oxidations in the sidechain[7,8,9] it seemed apparent that these oxidations were antagonised by the compound *in vivo*.

TABLE II

EFFECT OF SKF–A ON METABOLISM OF HEXOBARBITONE IN DOGS

Dogs received 30 mg./kg. hexobarbitone intraperitoneally. One week later experiment was repeated except that SKF 525–A was administered before the hexobarbitone injection.

Dog No.	SKF 525–A administered mg./kg.	Hexobarbitone half-life min.
1	0	90
	15	480
2	0	120
	15	720
	50	1200
3	0	810
	15	390

Previous work has demonstrated that one of the pathways of metabolism of the analgesic pethidine is through demethylation to yield norpethidine[10,11]. The effect of SKF 525–A on the metabolic transformation of pethidine was studied in mice. Table III shows that the total amount of the analgesic was higher in SKF 525–A treated than in untreated mice. Conversely, there was about five times as much of the

TABLE III

EFFECT OF SKF 525–A ON METABOLISM OF PETHIDINE IN MICE

Ten mice received 1·5 mg. of pethidine intraperitoneally. Five of these also received 50 mg./kg. SKF 525–A before the injection of pethidine. Animals were killed 60 minutes later and analysed for pethidine and norpethidine.

Without SKF 525–A		With SKF 525–A	
Pethidine mg./mouse	Norpethidine mg./mouse	Pethidine mg./mouse	Norpethidine mg./mouse
0·26	0·52	0·35	0·14
0·25	0·53	0·53	0·08
0·34	0·60	0·40	0·06
0·27	0·53	0·48	0·13
0·20	0·49	0·42	0·16
Average 0·26	0·53	0·44	0·11

demethylated product, norpethidine, in the untreated animals. These results point to a blocking of the demethylation of pethidine. Similarly, in the dog, the metabolism of amidopyrine, which is known to be demethylated to yield 4-aminoantipyrine *in vivo*[12], was antagonised by first giving the new compound (Fig. 1). From these and a number of similar experiments with other drugs it may be concluded that potentiating effects result from its inhibitory effects on the metabolism of a variety of drugs.

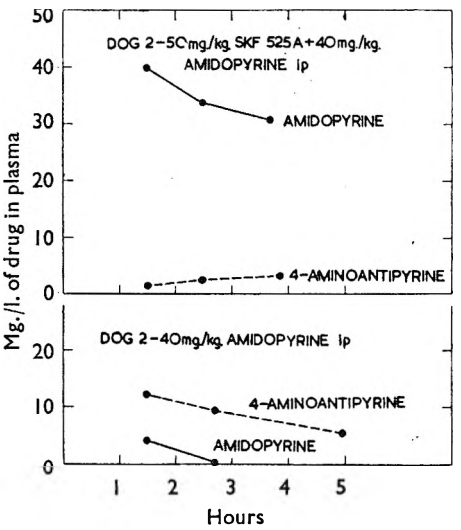


FIG. 1. Effect of SKF 525–A on the demethylation of amidopyrine in the dog.

At this point it might be desirable to clarify just what we mean by the term "potentiating" agent. SKF 525–A might better be called a prolonging agent since it acts only by interfering with the rate of metabolism of drugs. In contrast, the tranquillising agents, chlorpromazine and reserpine, which potentiate the action of hypnotics such as barbiturates and ethanol, are true potentiators since they do not affect the metabolism of these drugs but act by increasing the sensitivity of the central nervous system[13]. The difference between the actions of SKF 525–A and chlorpromazine was shown rather dramatically in the following way. When the former was given intravenously to mice and dogs which had just recovered from hypnosis induced by hexobarbitone, the animals were not visibly affected, since the compound could only slow down the biotransformation of the non-hypnotic amount of barbiturate remaining in the body. In contrast, if chlorpromazine were given to the awakening animals they reverted almost immediately to a deep hypnosis.

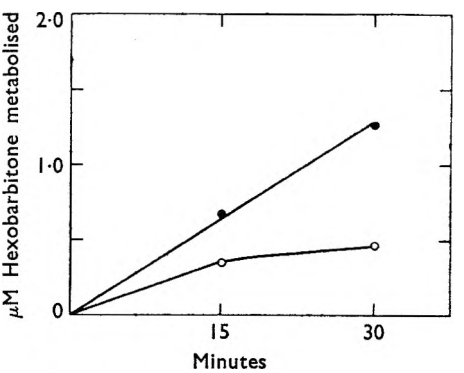


FIG. 2. Comparison of hexobarbitone metabolism in liver slices of control rats and rats pretreated with 25 mg./kg. SKF 525–A.
●——● Control.
○——○ SKF 525–A—treated.

To see how SKF 525–A affected a wide variety of drug metabolic pathways and to determine its mechanism of action at a tissue level we

turned to studies employing tissue slices and homogenates[14]. Liver slices of rats that had previously been injected with it were incubated with hexobarbitone. It is evident from the typical result shown in Figure 2 that hexobarbitone was metabolised considerably less rapidly in liver slices from these rats than in those of control animals. Since hexobarbitone is metabolised only by the liver[15] it is evident that SKF 525–A acts on this organ and this led us to examine its effects when added directly to liver slices.

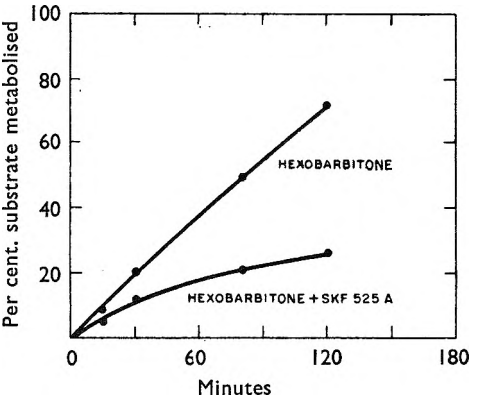


FIG. 3. Inhibition of hexobarbitone metabolism by SKF 525–A (2×10^{-4} M) added directly to rabbit liver slices.

SKF 525–A (2×10^{-4} M) added directly to liver slices was found to markedly inhibit the metabolism of hexobarbitone (Fig. 3) and other barbiturates such as pentobarbitone and quinalbarbitone. It also affected the biotransformation of a number of alkylamines including amidopyrine and pethidine (Fig. 4). Other drug metabolic pathways that the compound was shown to antagonise *in vitro* included deamination of amphetamine and splitting of the ether linkage in codeine to form morphine.

The effect of varying the concentration of SKF 525–A was determined. The influence of the inhibitor on hexobarbitone metabolism was evident at concentrations as low as 1×10^{-4} M and as the concentration of the

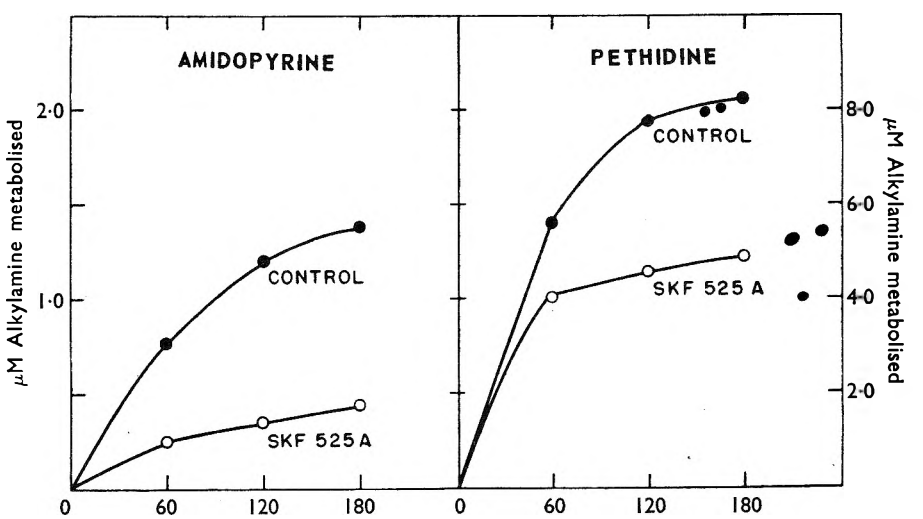


FIG. 4. Inhibition of amidopyrine and pethidine metabolism by SKF 525–A (2×10^{-4} M) added directly to rat liver slices.

former was increased there was a progressive increase in the degree of inhibition (Fig. 5). It is of interest that the simple acid component of SKF 525-A, diphenylpropylacetic acid (SKF acid), had inhibitory effects *in vitro* equal to those of the compound itself yet showed little effect *in vivo*, perhaps because of its rapid excretion or metabolic transformation.

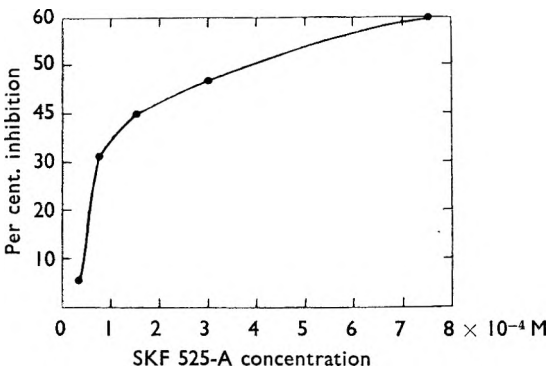


FIG. 5. Effect of various concentrations of SKF 525-A on hexobarbitone metabolism in rabbit liver slices.

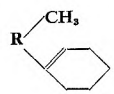
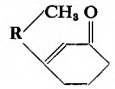
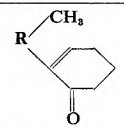
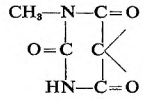
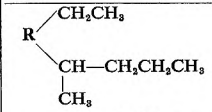
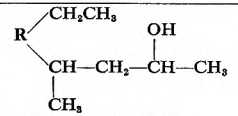
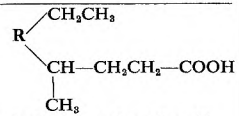
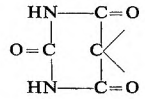
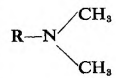
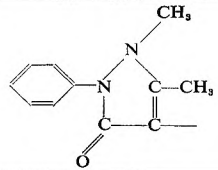
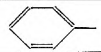
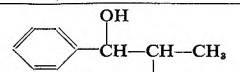
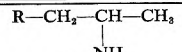
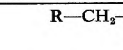
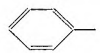
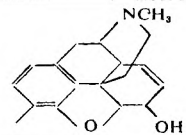
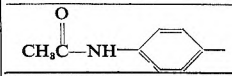
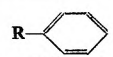
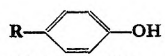
METABOLIC ROUTES

It seemed to us that if a number of drug metabolic pathways were susceptible to the same inhibitor they should have certain factors in common. Using liver homogenates, studies were undertaken to learn what these factors might be. A number of compounds, all oxidatively metabolised along a variety of metabolic pathways, were studied; and here, are described in detail our findings about the oxidation of hexobarbitone to ketohexobarbitone. In general, almost identical findings apply to a number of metabolic pathways including sidechain oxidation, dealkylation, deamination, ether cleavage and hydroxylation[16], examples of which are listed in Table IV.

Unfortified rabbit liver homogenates showed only slight activity in metabolising the drugs and this activity was completely lost after dialysis. Considerable activity was restored by the addition of nicotinamide and triphosphopyridine nucleotide (TPN), but diphosphopyridine nucleotide (DPN) could not replace TPN. Nicotinamide served presumably to protect TPN against enzymatic destruction[24]. Oxygen was also required, little drug metabolism being observed under anaerobic conditions. In addition, activity was enhanced by the addition of Mg^{++} and glucose-6-phosphate. The requirement for both TPN and oxygen first suggested that the various oxidative pathways in Table IV were carried out by the transfer of hydrogen from the various drugs, activated by dehydrogenase systems, with TPN acting as a hydrogen acceptor. This explanation, as we will see later, is far from being the correct one.

Having learned that the metabolic pathways depicted in Table IV all needed TPN, other requirements were sought that they might have in common. Rabbit liver homogenate was centrifuged at 9000 × g to separate the nuclei and mitochondria from the rest of the cell. The various drugs were metabolised in the supernatant fraction, but almost no activity was associated with nuclei or mitochondria. On centrifugation of the supernatant fraction at 80,000 × g the submicroscopic particles (microsomes) were separated from the soluble part of the cell. Neither

TABLE IV

Type of reaction	Substrate and structure	Products and structures			Structure of R group	Reference
Barbiturate side chain oxidation	 Hexobarbitone		or	 Ketohexobarbitone		15
	 Pentobarbitone	 Pentobarbitone alcohol	and	 Pentobarbitone carboxylic acid		17
Dealkylation	 Amidopyrine	$R-NH_2$ 4-aminoantipyrine	and	2HCHO Formaldehyde		18
	$R-NH-CH_2CH_3$ Monoethylaniline	$R-NH_2$ Aniline	and	CH_3CHO Acetaldehyde		19
	$R-NH-CH_3$ Ephedrine	$R-NH_2$ Norephedrine	and	HCHO Formaldehyde		20
Deamination	 Amphetamine	 Phenylacetone	and	NH_3 Ammonia		21
Ether Cleavage	$R-O-CH_3$ Codeine	R—OH Morphine	and	HCHO Formaldehyde		22
	$R-O-CH_2CH_3$ Phenacetin	R—OH *p*-Hydroxyacetanilide	and	CH_3CHO Acetaldehyde		22
Hydroxylation	 Aniline or acetanilide	 *p*-Hydroxyaniline	or	*p*-Hydroxyacetanilide	NH_2- Aniline or $CH_3-C(=O)-NH-$ Acetanilide	23

of these fractions by itself possessed any activity but when added together the recombination was about as effective as the original whole homogenate (Table V). It was evident that factors present in both microsomes and soluble supernatant were necessary to carry out the metabolism of the various drugs. Activity was increased if more microsomes were added to the mixture but maximal effect was still retained even in the presence of only a small volume of soluble fraction. It seemed reasonable to presume, therefore, that the various enzymatic mechanisms were localised in microsomes and that the soluble fraction supplied something, normally present in excess, that they all needed. The finding, previously mentioned, that the activity of dialyzed homogenates was enhanced by the addition of Mg^{++} and glucose-6-phosphate, together with the known presence of considerable glucose-6-phosphate dehydrogenase in the soluble fraction, suggested that the form of TPN required in the complete system was actually TPNH, generated through the following reaction:

TABLE V

CELLULAR LOCALISATION OF ENZYME ACTIVITY

Various cell fractions were prepared from rabbit liver by differential centrifugation and incubated 1 hour at 37° with 1·0 μM of hexobarbitone at pH 7·4.

	Hexobarbitone metabolized μ moles
Whole homogenate	0·50
Supernatant fraction	0·65
Microsomes	0
Soluble fraction of supernatant ..	0
Microsomes + soluble fraction ..	0·70

$$\text{glucose-6-phosphate} + \text{TPN} \xrightarrow{\text{glucose-6-phosphate-dihydrogenase}} \text{phosphogluconic} + \text{TPNH}$$

Substantiation of this hypothesis was afforded by the finding that all the substrates in Table IV could be metabolised by washed microsomes with the soluble fraction substituted by a TPNH generating system consisting of glucose-6-phosphate dehydrogenase, glucose-6-phosphate and TPN. Direct evidence that TPNH was involved in these reactions was obtained by the demonstration that the addition of chemically prepared TPNH to washed microsomes could replace the soluble fraction (Table VI). Thus we find additional factors possessed in common by a variety of drug metabolic pathways—localisation in liver microsomes and a requirement for TPNH and oxygen.

Now, instead of the familiar picture of oxidation proceeding through dehydrogenation with DPN or TPN acting as hydrogen acceptors, we are faced with a strange kind of oxidation where the pyridine nucleotide is already in a reduced form and therefore unable to accept hydrogen. Obviously oxidation must be proceeding in an unusual way.

Some progress has been made towards the elucidation of the mechanism of these oxidative enzymes. Drs. La Du and Gillette have obtained important information about the role of TPNH[25]. They have demonstrated the presence of an oxidase (TPNH oxidase) in liver microsomes, which catalysed the oxidation of TPNH to TPN even in the absence of drug substrates. This reaction was not blocked by cyanide, showing that

the normal cytochrome system was not involved in this hydrogen transfer. Material with hydrogen peroxide-like properties was demonstrated to be a product of the TPNH oxidation—suggesting that hydrogen peroxide or a peroxide-like intermediate was utilised by a variety of enzymes in microsomes to carry out the various drug reactions. Although other tissue cells were shown to contain microsomes with TPNH oxidase

TABLE VI

REQUIREMENTS FOR TPNH IN OXIDATION OF HEXOBARBITONE BY LIVER MICROSOMES

Microsomes were incubated for 1 hour with 1 μmole of hexobarbitone at pH 7·4.

Components	Hexobarbitone oxidised μ moles
Microsomes + 1·0 μmole TPN	0
Microsomes + 1·0 μmole + 4 μmoles glucose-6-phosphate	0
Microsomes + 1·0 μmoles TPN + 4 μmoles glucose-6-phosphate + glucose-6-phosphate dehydrogenase	0·30
Microsomes + TPNH	0·61
Microsomes + 0·5 ml. soluble fraction + 1 μmole TPN	0·57

activity, only those in liver catalysed the various metabolic pathways outlined in Table IV, thus confirming the generally considered view that drug metabolism usually occurs mainly in liver. Apparently the liver microsomes contain additional catalysts which the microsomes of other tissues lack in order to effect the metabolism of drugs.

Before leaving the subject of the role of the liver in drug metabolism, mention should briefly be made of some preliminary work in our laboratory. We read in the textbooks that the liver is responsible for the metabolism of many drugs; therefore, subjects with liver disease probably present a therapeutic problem. This may not be true with most drugs. Drs. Burns and Weiner showed that subjects with badly diseased livers could still metabolise antipyrine, salicylate, phenylbutazone, amidopyrine, pethidine, pentobarbitone and a number of other drugs as readily as did normal subjects. Thus, the fact that the liver does not function in a normal manner does not necessarily mean that the enzyme systems involved in drug metabolism are impaired.

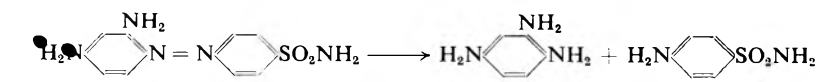
A number of oxidative pathways of drug metabolism including side-chain oxidation, dealkylation, deamination, ether cleavage and hydroxylation all require TPNH and oxygen and are harboured in the microsomes of the liver cell. These pathways may have a step in common—the production of a peroxide-like intermediate—which under the influence of other catalysts in liver microsomes is utilised in the biotransformation of the various types of drugs.

But in considering the mechanism of drug oxidation we are left with a number of unsolved problems. Does each type of metabolic pathway in Table IV involve the same enzyme system? This is of course extremely unlikely and, in fact, a number of observations indicate that this is not so. For example, microsomes prepared at pH 7·0 rapidly lose their ability to hydroxylate aniline and acetanilide but not to dealkylate amidopyrine[23], indicating that the hydroxylation mechanism is more labile than the dealkylation one. Although it may be assumed that the different

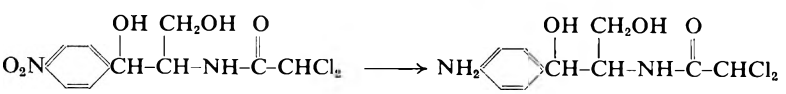
metabolic pathways require different enzyme systems, does the demethylation of methylamines require only a single system, and can this system also dealkylate ethyl and propylamines? Again, although the evidence indicates that the various types of reactions require a common peroxide-like intermediate, there is no definitive information concerning the nature of the reaction of this substance with drugs. A main difficulty in learning more about the mechanisms lies in the lability of the various enzyme systems which so far have suffered inactivation when microsomes are subjected to various solubilisation procedures.

Previous work from this laboratory has shown that at least two of the metabolic pathways in Table IV, hydroxylation and ether cleavage, can occur in the presence of a model system consisting of ascorbic acid, oxygen and inorganic iron chelated with versene[26]. There is evidence that the action of this system is mediated through the generation of hydroxyl groups produced by a reaction product of hydrogen peroxide with ascorbic acid[27]. Will sidechain oxidation, dealkylation and deamination also occur in the presence of this system? If so, this would suggest that the various oxidative pathways can occur *in vitro*, non-enzymatically, with hydroxylation as a common step. Although the reactions *in vivo* require enzymes, we are looking into the possibility that a common step in the microsomal oxidation of drugs may be the production of hydroxyl groups.

Another unsolved puzzle is how SKF 525–A acts. This problem has taken an unusual turn. The original premise that led to our studies on the oxidative drug enzymes was that since SKF 525–A inhibited so many pathways of drug metabolism, these must have certain factors in common. An obvious thought is that the compound blocks the oxidation of TPNH. But this step is not affected by the inhibitor[25]. Furthermore, there are a number of instances where the metabolism of a drug which follows one of the pathways outlined in Table IV is not antagonised by SKF 525–A. The demethylation of methylaniline to aniline which is catalysed by an enzyme system in microsomes and requires TPNH and O_2 is not affected by the inhibitor even at high concentrations[19], inhibition not even being restricted to oxidative pathways. Thus, Dr. Fouts has shown that the enzymatic reductions of prontosil and chloramphenicol in rabbit liver homogenates are also inhibited by SKF 525–A[28].



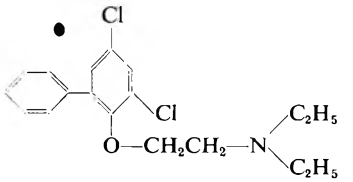
Prontosil — Sulphanilamide



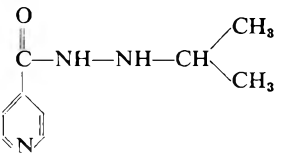
Chloramphenicol — Reduced chloramphenicol

These reactions also require TPNH but proceed anaerobically. Still more surprising is the finding that SKF 525–A inhibits drug enzymes in microsomes that do not even require TPNH, for example the formation of a glucuronide with morphine[14] and the de-esterification of procaine[29].

To further complicate the story, Dr. Fouts has shown that Lilly 18947[30] and Marsilid[31], compounds structurally unrelated to SKF 525–A, block



Lilly 18947
2,4-dichloro-6-phenylphenoxy-ethyl diethylamine



Marsilid
2-*iso*propyl-1-*iso*nicotinyl hydrazide

the same drug enzyme systems. The possibility is entertained that the inhibitors act in some physico-chemical manner perhaps by preventing, in some way, the entrance of certain drugs into microsomes.

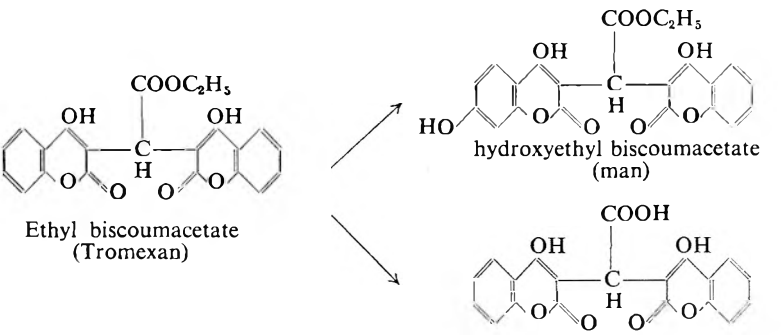
Species Differences and their Relation to Drug Metabolism

Species differences are of some concern to the pharmacologist who screens drugs in animals and on the basis of the results so obtained has to decide which ones to try in man. It is well known that various animal species react differently to the same drug. Some of the differences are mainly qualitative in nature; for instance, morphine depresses man but stimulates racehorses and cats. It is probable, however, that most species differences are in the duration of action of drugs and here, a few examples would be pertinent.

Phenylbutazone, an antirheumatic drug, is metabolised unusually slowly in man, about 10 to 15 per cent. per day[32] but in mice, rabbits, dogs, guinea-pigs and horses it disappears from the body in a few hours. It is not surprising that the antirheumatic action of this compound was first observed in man by chance, since relatively enormous doses are needed, because of its rapid metabolism, to show an anti-inflammatory effect in rats.

Pethidine, a narcotic analgesic, is metabolised in man at the rate of about 17 per cent. per hour, a single dose exerting an analgesic effect lasting three to four hours. In a study of its fate and distribution, Dr. Burns in our laboratory decided to give a dog 20 mg. per kg., which in man would be considered a huge dose. He was prepared for artificial respiration since it was fully expected that the animal would experience dire effects including loss of breathing. The drug was infused over a period of 20 minutes following which, to his surprise, the dog leaped from the table and walked away. The explanation for the relative immunity to the effects of the narcotic lies in the extraordinarily rapid rate of biotransformation in the dog, 70 to 90 per cent. per hour. The rapid disappearance of pethidine may also explain the difficulty of producing either tolerance or addiction to the drug in dogs. One wonders how many useful analgesics are still sitting neglected on the dusty shelves of pharmaceutical houses because they showed only minimal effects on test animals, merely because they were metabolised too rapidly in the particular species in which they were tested.

Another example of species differences may be seen with ethyl biscoumacetate, an anticoagulant similar in structure to dicumarol. It was introduced as a safer anticoagulant on the basis of its rapid metabolism compared to dicumarol, so that recovery from bleeding difficulties due to overdosage would also be rapid. It so happens that ethyl biscoumacetate was first screened in the rabbit, an animal that metabolises the drug at the same rate as in man. But this was sheer coincidence since the two species metabolise the drug entirely differently, man, by hydroxylation of an aromatic ring and the rabbit by de-esterification.



If the drug had been screened in dogs, it would probably have been discarded since in this species it is metabolised almost as slowly as dicumarol, about 3 per cent. per hour.

One of the most dramatic examples of species differences was uncovered by us in a programme in which, in collaboration with Mr. William Lott of E. R. Squibb, we were trying to develop a barbiturate that would be rapidly metabolised in man. One of the compounds investigated, a bromo*cyclo*hexenyl derivative of barbituric acid (thialbarbitone without the bromine atom), when given to dogs, produced a deep anæsthesia. But no matter how long the dogs were kept under anæsthesia, by intermittent infusion of the drug, the animals showed almost complete recovery shortly after termination of the infusion. Plasma level measurements showed that the drug was metabolised in dog at an enormous rate compared to thiopentone. Very hopefully, thinking that,

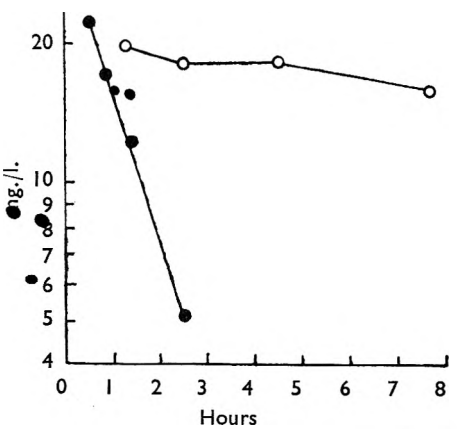


FIG. 6. Comparison of plasma levels of a bromo*cyclo*hexenyl derivative of barbituric acid in man and dog.

○——○ Man (dose 15 mg./kg.).
●——● Dog („ „).

at long last, we might have discovered the intravenous anæsthetic of choice, the compound was administered in man only to find that it was one of the most stable barbiturates we had ever studied (Fig. 6).

These examples indicate in a general way the importance of drug metabolism in causing species differences. These differences were studied in a more detailed fashion by comparing the duration of action and the rate of biotransformation of hexobarbitone in a number of animal species. The duration of action of the barbiturate bore an inverse relationship to its rate of disappearance from the body (Table VII). The drug was

TABLE VII

SPECIES DIFFERENCES IN METABOLISM OF HEXOBARBITONE
DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each species.

	Sleeping time minutes	Hexobarbitone half-life minutes	Enzyme activity μg./g./hr.
Mice (12) ..	12 ± 8	19 ± 7	598 ± 187
Rabbits (9) ..	49 ± 12	60 ± 11	294 ± 28
Rats (10) ..	95 ± 15	139 ± 54	134 ± 51
Dogs (5)* ..	315 ± 105	261 ± 20	36 ± 29

* Dogs received 50 mg./kg. of hexobarbitone.

metabolised extremely rapidly in the mouse having a half-life of only 15 minutes. In other animal species it was metabolised more slowly and in dog it was found to be relatively stable with a half-life of over 300 minutes (about the same as in man). Although the mouse, in general, metabolises hexobarbitone and a number of other drugs more rapidly than do other animal species, there is no clear-cut relationship between the size of the species and the rate of biotransformation. For example, the rat, though smaller than the rabbit, inactivates hexobarbitone more slowly.

Since Nature has packaged a number of her drug enzyme systems in microsomes, they can be easily separated from the rest of the cell. It became possible, therefore, to ascertain directly whether species differences with hexobarbitone could be explained in terms of the activity of an enzyme system in microsomes. When the activity of the hexobarbitone enzyme system in microsomes was assayed it, also, was found to bear an inverse relationship to the duration of drug action. For example, the activity of the hexobarbitone oxidising enzyme system in mouse microsomes was about 17 times that in the dog (Table VII). Thus with hexobarbitone we have demonstrated that species differences in duration of action can be expressed in terms of the activity of a single enzyme system, the microsomal system which "detoxicates" it. We now have accumulated evidence that species differences can be similarly explained in terms of a single "detoxication" enzyme system with a number of other drugs.

As though species differences are not enough trouble to the pharmacologist, strain differences must also be considered. We were fortunate in having available a number of inbred strains of mice. Individual mice of a given strain given a 100 mg. per kg. of hexobarbitone slept for a

remarkably uniform interval of time. But different strains slept for different times varying from an average of 12 minutes in strain SWR/HEN to 55 minutes in strain 1/LN.

In addition there are sex differences. It has been known for a number of years that female rats anæsthetised with certain barbiturates slept considerably longer than did males[34,35]. While certain important differences between the two sexes have long been recognised and investigated in some detail, this manifestation seemed rather curious and worth while studying.

TABLE VIII

SEX DIFFERENCES IN THE METABOLISM OF HEXOBARBITONE IN RATS
DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each series.

Sex	Sleeping time minutes	Plasma level at 60 minutes μg./ml.	Enzyme activity μg./g./hr.
Females	90 ± 15 (10)	65 ± 8	134 ± 51
Males	22 ± 5 (11)	23 ± 9	682 ± 102

TABLE IX

INFLUENCE OF ŒSTRADIOL ON THE METABOLISM AND DURATION OF ACTION OF HEXOBARBITONE IN MALE RATS

DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each series.

	Sleeping time minutes	Plasma level at 60 minutes μg./ml.	Enzyme activity μg./g./hr.
Control	22 ± 5 (11)	23 ± 9	682 ± 102
Œstradiol ..	84 ± 22 (9)	71	177 ± 33

When male and female rats were given 100 mg. per kg. of hexobarbitone, the females slept about four times as long as the males. A comparison of the plasma levels indicated that the drug was metabolised much more rapidly in males than in females. In accord with this finding, microsomes isolated from livers of male rats showed considerably higher enzyme activity than those of females in oxidising hexobarbitone (Table VIII). Thus we see that the female rats were not actually more susceptible to hexobarbitone but were merely unable to metabolise it as rapidly.

The observed sex differences prompted us to investigate the effects of the sex hormones. Male rats were given œstradiol for several weeks before the administration of a single dose of hexobarbitone. The treated rats now slept much longer than the controls, in fact, as long as the females. Studies with liver microsomes showed that these had lost much of their capacity to metabolise hexobarbitone (Table IX). Female rats were given testosterone for two weeks and then given a single dose of hexobarbitone. Now when given hexobarbitone they slept as short a time as did males. Correspondingly, their liver microsomes had acquired considerably more capacity to metabolise hexobarbitone (Table X).

When recounting these experiments at a gathering of anæsthesiologists, I was told emphatically that they had no clinical impression of any such differences in the reaction of males and females of the human species to barbiturates. We therefore decided to compare the duration of action of hexobarbitone in males and females of a number of other species including

TABLE X

INFLUENCE OF TESTOSTERONE ON THE METABOLISM AND DURATION OF ACTION OF HEXOBARBITONE IN FEMALE RATS

DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each series.

	Sleeping Time minutes	Plasma level at 60 minutes μg./ml.	Enzyme activity μg./g./hr.
Control	90 ± 15 (10)	65 ± 8	134 ± 51
Testosterone ..	38 ± 17 (14)	37 ± 14	543 ± 123

mice, guinea-pigs, rabbits and dogs. To our consternation no differences between the sexes were observed in these animals. Furthermore, œstradiol and testosterone could not induce any appreciable change in the detoxification rate of the barbiturates in mice and rabbits. Just why this sex difference seems to be present only in rats and whether it extends to other drugs we do not yet know. But the considerable amount of basic research with hormones that has been made with the rat gives one considerable food for thought. This animal behaves differently in this respect, might it not in others also?

THE NORMAL ROLE OF THE ENZYME SYSTEMS DISCUSSED

When we first began our studies on drug metabolism we shared the traditional concept that foreign compounds were metabolised by mechanisms which acted on structurally related substances normally present in the body. When our studies made it evident that the body again and again was able to "detoxicate" compounds it has never "seen" before, we began to question this concept. A most striking property of most enzymes is not only their catalytic effect on chemical reactions but also their specificity; that is, a given enzyme can usually catalyse only a small range of reactions and in many cases only a single reaction. After all, if every foreign compound which even faintly resembled a substrate in the body were acted on by the same enzyme system a normal function of the body might soon be overwhelmed in the presence of a foreign compound. Consequently, some of us are gaining the viewpoint that the microsomal enzyme systems in liver may be there just to "detoxicate" foreign compounds. For this function, enzyme systems would need to be extremely nonspecific; one to dealkylate alkylamines, another to hydroxylate aromatic compounds, and so forth.

Indirect evidence is gradually accumulating for the point of view that the microsomal drug enzymes metabolise only foreign compounds. For one thing, Mr. Gaudette in our laboratory found that the normally

occurring alkylamines, such as sarcosine and dimethylaminoethanol, were not demethylated by the microsomal dealkylation system. Yet sarcosine is readily demethylated by an enzyme system present in mitochondria[36] which will not dealkylate foreign alkylamines. Again, Mitoma and Udenfriend[23] showed that a considerable number of normally occurring aromatic compounds, for example, tryptophan and phenylalanine, were not hydroxylated by the hydroxylation system in microsomes but required specific mechanisms present in another part of the cell[37,38]. In what way are these compounds protected from the microsomal system? If we think of the microsomes as particles with a membrane which will, ordinarily, pass non-polar compounds but not polar compounds, we have a plausible picture of the way the body protects its essential substrates from wasteful metabolism due to the non-specific microsomal enzymes. Thus far, I might stress, we have found no normal substrate which is metabolised by the drug enzyme systems in microsomes.

Mr. Gaudette also demonstrated that amidopyrine was not appreciably dealkylated in liver homogenates of turtles, frogs and goldfish and hexobarbitone was not oxidised in liver homogenates of turtles and frogs. In fact, in preliminary experiments, frogs anæsthetised with hexobarbitone excreted it mainly in an unchanged form. The evidence is certainly incomplete, but it is possible that the microsomal drug enzyme systems are absent in lower animals.

Thus, we can consider the possibility that the enzyme systems in microsomes of mammals which have a major role in limiting the duration of action of drugs have developed by a process of evolution to protect the organism from a multitude of foreign compounds ingested in food as well as produced by micro-organisms in the intestines.

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RESEARCH PAPERS

A METHOD FOR THE DETERMINATION OF pA_2 AT TWO MINUTES

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THE systematic quantitative study of the actions of drugs on specific drug receptor surfaces in isolated tissues was initiated in this country by A. J. Clark. Work by Clark and his collaborators[1–4] and by Gaddum[5,6] established the existence of a number of different types of cell-surface receptors. Each type was specifically fitted by a naturally occurring key or activating drug, of high potency, capable of producing characteristic action on the tissue. The perfect fit of the key drug for the lock or receptor was believed to result from the chemical configuration of both, and it was therefore expected that the affinity between drug and receptor would be higher for the key than for any other drug. Key drugs appeared to occupy their receptors reversibly and according to the law of mass action, and the tissue responses evoked were considered proportional to the percentage of specific receptors occupied. In accordance with theory, drugs were found amongst compounds chemically related to key drugs, which themselves produced no action on the isolated tissues, but which made these tissues less sensitive to the action of the related key drug. These inhibitor compounds were apparently attracted to the receptors and occupied them, less adequately than the key drug perhaps since no tissue activation occurred, and usually less reversibly than the key drug. When such an inhibitor and the key drug were allowed to act on the tissue together, both competed for the receptors, and did so according to the law of mass action; therefore, a higher concentration of key drug was required to produce a given effect in the presence of the inhibitor than in its absence. Such specific competitive antagonists of key drugs have already proved their value clinically in combating, for instance, the unwanted effects of naturally occurring key drugs, such as histamine, acetylcholine, and adrenaline.

Schild[7] introduced pA_x as a measure of the strength with which such an inhibitor drug competes with a key drug in any one isolated system. He defined pA_x as "the negative logarithm to the base 10 of the molar concentration of an antagonistic drug which will reduce the effect of a multiple dose, x, of active drug to that of a single dose." Since the effect of these inhibitor drugs varies with time, the value pA_x is determined after a stated interval of contact between tissue and inhibitor drug, usually 2 minutes.

The reliability of this pA scale for assessment of the relative potency of synthetic inhibitor drugs is so widely accepted that need has arisen for a

simple quick method of pA_2 ($x = 2$) determination at 2 minutes, which allows the calculation of limits of error from the internal data of the experiment. The object of this paper is to present such a method. A detailed description of the method is given first, and this is followed by evidence which must be provided in support of its validity.

The Method

A strip of guinea-pig ileum is suspended in oxygenated Tyrode's solution at a temperature between 32° and 34°C., in a bath of known and constant volume. A suitable dose, k, of key drug is found by trial; this dose should produce an effect which is approximately 50 per cent. of the maximum. Dose k is repeated every 3 minutes, and is allowed to act for a fixed contact period, e.g., 30 seconds before the bath fluid is changed. When the response to k has been constant, or nearly so, for three consecutive contractions, a dose of inhibitor is added to the bath exactly 2 minutes before the next dose of key drug is due. At the end of this 2 minutes, double the usual dose, $2k$, of key drug is added without washing out; it is allowed to exert its effect in the presence of the inhibitor throughout the usual contact period for key drug; then the two are washed out together. Return is then made to the original dose, k, of key drug; it is added to the bath every 3 minutes for the same fixed contact period as before, until the tissue recovers from the effect of the inhibitor, and settles down again to a constant response. Then, after 3 control contractions in response to dose k, the inhibitor drug may be tested again.

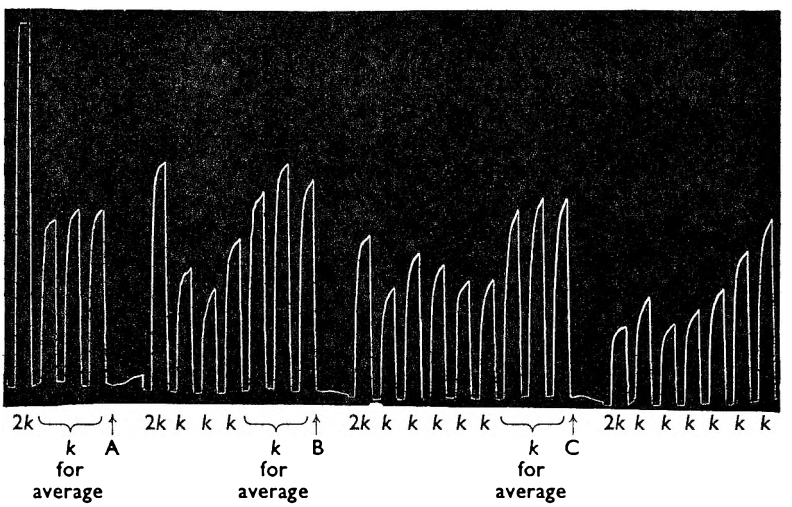

FIG. 1. Determination of pA_2 at 2 min. The tracing was made by guinea-pig ileum contracting in a 20 ml. bath of Tyrode's fluid at 32° C. See text for explanation.

After a few preliminary trials, three doses, A, B, and C of inhibitor are selected. These doses A, B, and C are chosen as weights of inhibitor which increase in series as simple powers of 2 or 3. The effect of dose $2k$ of key drug after A should be greater than that of k, and less than that of

$2k$ in the absence of inhibitor. The effect of dose $2k$ after C should be less than that of k in the absence of inhibitor, but must be readily measurable. B will produce an inhibition of $2k$ intermediate between those due to A and C.

Doses A, B, and C of inhibitor are then given, exactly as described above, in the order of a 3 × 3 Latin Square (e.g., ABC, BCA, CAB).

The procedure is illustrated in Figure 1. The tracing is part of a record obtained by a technician who was asked to estimate pA_2 at 2 minutes for histamine-diphenhydramine, and who was given only the above written instructions. The doses selected were :—k, 1 μg. histamine; A, B, and C, 0·07, 0·14, and 0·28 μg. of diphenhydramine respectively (these increase as simple powers of 2). The bath was calibrated to a volume of 20 ml. The drum was stopped each time when the key drug was washed out, and was restarted either 30 seconds before the addition of key drug, or, when inhibitor was used, 30 seconds after its addition to the bath. The figure shows the first line of a completed Latin Square. The first response recorded is to $2k$, the second to k, in the absence of any inhibitor. Three control responses to k were followed by the addition of A to the bath. A small spontaneous contraction occurred during the 2 minutes contact of the tissue with diphenhydramine before the addition of $2k$ to the bath and was ignored. Whereas the three control responses to k were satisfactory before the addition of inhibitor doses A and C, they were not so before the addition of B; the tissue had probably not fully recovered from the action of A by the first of the three control doses of k, and had not settled down to give a constant, or nearly constant, response to k.

When the whole Latin Square had been completed the heights of the relevant contractions were measured. On each occasion when diphenhydramine had been used, the difference between the height of the response to $2k$ and the average height of the three preceding control responses to k was expressed as a plus or minus percentage of that average. The results obtained are shown in Table I.

TABLE I

SUMMARY OF RESULTS FROM A SINGLE DETERMINATION OF pA_2 AT 2 MINUTES FOR DIPHENHYDRAMINE-HISTAMINE ON GUINEA-PIG ILEUM

Dose of diphenhydramine	μg.	Effect of diphenhydramine $\frac{(100(2k-k))}{k}$			
		Individual trials			Average
A	0·07	+30	+23	+16	+23
B	0·14	−23	−34	−33	−30
C	0·28	−60	−74	−70	−68

k = average control response to 1 μg. histamine; $2k$ = response to 2 μg. histamine in the presence of diphenhydramine.

The pA_2 value for histamine-diphenhydramine at 2 minutes was then determined both graphically, and by calculation.

pA_2 determined graphically. The average percentage differences between the responses to $2k$ after and k before diphenhydramine (Table

I) were plotted as ordinates against the logarithms of the doses of antihistamine as abscissæ. The line of best fit for the resulting 3 points was drawn by eye (Fig. 2); then the log-dose corresponding to zero percentage difference was read from the graph (2·91). Its antilogarithm (0·096) gave the μg. of antihistamine which, when added to the bath volume (V = 20 ml.), would have produced a molar concentration the negative logarithm of which was pA_2 diphenhydramine-histamine at 2 minutes.

The molarity resulting from the addition w μg. of drug to a bath volume of V ml. is given by the following equation:—

$$\text{Molarity} = \frac{w}{\text{M.W.} \times \text{V} \times 1000}, \text{ where M.W. stands for molecular weight.}$$

Hence the molarity resulting from the addition of 0·096 μg. of diphenhydramine (M.W. 255) to a bath volume of 20 ml. was $1{\cdot}87 \times 10^{-8}$. The pA_2 value required was given by the negative logarithm of this figure. A negative logarithm of any number is the logarithm of the reciprocal and so can be found by subtracting the logarithm of the original number from the logarithm of 1·0. The value for pA_2 diphenhydramine-histamine at 2 minutes given by this experiment was, therefore, $0{\cdot}0000-\bar{8}{\cdot}2724 = 7{\cdot}7$.

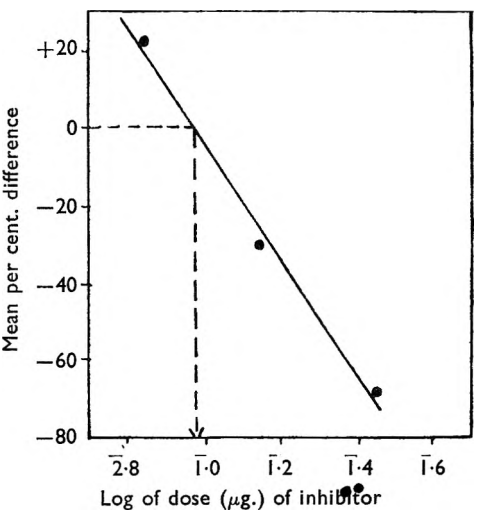

FIG. 2. Graphical determination of the log-dose of inhibitor which, when added to the bath volume, will, in two minutes, reduce the effect of a double dose of activating drug to that of a single dose. Ordinates: average percentage difference between responses to a double dose of activating drug in the presence of inhibitor, and to a single dose of activating drug in the absence of inhibitor.

pA_2 by calculation. The first object of this procedure was to find the line of best fit for the 3 points in Figure 2 exactly, instead of approximately by eye. The method used was the standard one for the calculation of regression lines where the response is graded. Its application is briefly explained.

The equation for any straight line is: $y = a + bx$. (1) a and b are constants; b gives the slope of the line. y refers to the response and is dependent on x, the log-dose.

Let Σ stand for "the sum of", and $\bar{x}$ and $\bar{y}$ for the means of all values for x and y respectively.

Since the line of best fit for the 3 points will pass through the means $\bar{x}$ and $\bar{y}$ with a slope b, and will be such that $(\bar{y}-y) = O$, and $(\bar{y}-y)^2$ is a minimum, $b = \dfrac{(\bar{x}-x)(\bar{y}-y)}{(\bar{x}-x)^2}$. The calculation of $\bar{x}$, $\bar{y}$, and b was most

easily made in tabular form (Table II). In order to reduce the arithmetic the actual doses of inhibitor used are multiplied by some suitable fraction to convert them to 2, 4, and 8 or 3, 9 and 27. In this case the doses of diphenhydramine in μg. (column 1) were first multiplied by 100/3·5 (column 2), and were then converted to logarithms to the base 2, giving

TABLE II

CALCULATION OF THE LINE OF BEST FIT FROM THE DATA IN TABLE I

Dose of diphenhydramine		μg. × 100/3·5 as log. to base 2·0	Response per cent. change	Calculation of slope (b)				
μg.	μg. × 100/3·5	x	y	$\bar{x}-x$	$\bar{y}-y$	$(\bar{x}-x)(\bar{y}-y)$	$(\bar{x}-x)^2$	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	column
0·07	2·0	1	+23	+1	−48	−48	+1	
0·14	4·0	2	−30	0	+5	0	0	
0·28	8·0	3	−68	−1	+43	−43	+1	
		$\Sigma x = 6$	$\Sigma y = -75$			$\Sigma = -91$	$\Sigma = +2$	
		$\bar{x} = 2$	$\bar{y} = -25$					

$$b = \frac{(\bar{x}-x)(\bar{y}-y)}{(\bar{x}-x)^2} = \frac{91}{2} = -45{\cdot}5$$

$\bar{y} = a + b\bar{x}$, therefore $a = 66{\cdot}0$.

values for x (column 3). The corresponding values for y, which were the averages of the percentage differences between the responses to $2k$ and k at each dose level of diphenhydramine (Table I) are listed in column 4. $\bar{x}$ and $\bar{y}$ are calculated in columns 3 and 4 respectively and b in columns 5 to 8. b is given by the sum of column 7 divided by that of column 8.

These calculated values for $\bar{x}$, $\bar{y}$, and b were then used to find the value for a, by substitution into equation (1).

Since a and b were now known, the value of x corresponding to any given value of y could be found from equation (1). The value of x required for the estimation of pA_2 was that when $y = O$; i.e., $x = 1{\cdot}451$, which is equivalent to $2^{1{\cdot}451} \times \frac{3{\cdot}5}{100}$ μg. To evaluate $2^{1{\cdot}451}$, let z be the logarithm of the required number to the base 10, then $z = \log_{\cdot 10} 2{\cdot}0 \times 1{\cdot}451 = 0{\cdot}3010 \times 1{\cdot}451 = 0{\cdot}4368$, antilogarithm 2·734. Then the required value of x in μg. was $2{\cdot}734 \times \frac{3{\cdot}5}{100} = 0{\cdot}9560$. The molarity which resulted from the addition of 0·9560 μg. of diphenhydramine to the bath, and the pA_2 value for diphenhydramine-histamine at 2 minutes were then estimated exactly as described in the graphical method. The pA_2 value at 2 minutes obtained by calculation was 7·73.

The error of this estimate was most easily calculated in tabular form (Table III). The values of x (log-dose) and the corresponding observed values of y (average percentage responses) were entered in columns 1 and 2 respectively. The values of y given by the line of best fit were then calculated by substitution into equation (1), and were entered in column

3. Column 4 gave the difference between the observed and calculated values for y and column 5 these differences squared and multiplied by the number (n) of observations averaged to give the observed value of y. The variance (s^2) was obtained by dividing the total sum of squares in column 5 by N-5, where N stands for the total number of trials (5). This reduction of 5 was made because repeated trial had shown the variances so computed barely larger than those obtained by full analysis. It follows from equation (1) that there must be two components of the standard deviation of any value for y, because both a and b may be in error; moreover, any error in b will become magnified as the value for x departs from $\bar{x}$. Since the variance of $b = \frac{s^2}{\Sigma n(\bar{x}-x)^2}$ and the standard error of the mean $= \sqrt{s^2/N}$, the standard deviation (s_y) of any value of y was given by the equation $s_y = s\sqrt{\frac{1}{N} + \frac{(\bar{x}-x)^2}{\Sigma n(\bar{x}-x)^2}}$. It remained only to determine the standard deviation of y at the dose level x corresponding to pA_2 at 2 minutes. When $x = 1{\cdot}451$, $s_y = 4{\cdot}26$.

TABLE III

CALCULATION OF THE ERROR OF THE ESTIMATE OF pA_2 AT 2 MIN.

x	y obs. y_o	y calc. y_c	Difference $y_o - y_c$	Difference$^2 \times n$ $n(y_o - y_c)^2$
(1)	(2)	(3)	(4)	(5)
1	+23	+20·5	+2·5	18·75
2	−30	−25·0	−5·0	75·00
3	−68	−70·5	+2·5	18·75
				$\Sigma = 112{\cdot}5$
				$s^2 = 28{\cdot}125$

Applying fiducial limits this value for s_y was multiplied by the value of t with 4 degrees of freedom at a probability level of 0·95 ($t = 2{\cdot}776$ and $s_y.t = 11{\cdot}83$). The fiducial limits of y when $x = 1{\cdot}451$ were, therefore, $\pm 11{\cdot}83$. Values of x corresponding to these limiting values of y were then calculated by substitution into equation (1); i.e., $x = 1{\cdot}7105$ or $1{\cdot}1905$. The corresponding pA_2 values were calculated as above, and were found to be 7·65 and 7·81, differing from the mean pA_2 value of 7·73 by 0·08 or 1·03 per cent. The fiducial limits (P = 0·95) were therefore $\pm$ 1·03 per cent.

EXAMINATION OF THE METHOD

The method described is valid only because the percentage reduction in the response to $2k$ of key drug in the presence of inhibitor is linearly related to the log-dose of inhibitor for a range exceeding 90 per cent. of the uninhibited response to $2k$. Evidence of this fact was obtained in the course of 21 estimations of pA_2 at 2 minutes for antihistamine in extracts. These estimates were made by the method advocated above except that uninhibited responses to $2k$ were recorded amongst the control responses to k. The percentage differences between the responses to $2k$ before and after antihistamine were plotted as abscissæ, and the differences between the observed and calculated values for y as ordinates. The scatter diagram (Fig. 3) which resulted showed first, that the deviation of any point from linearity never exceeded 10 per cent. of y; secondly, that 86 per cent.

of all points deviated from linearity by less than 5 per cent. of y; thirdly, that departure from linearity was not increased, but tended to decrease, at the limits of the working range (5 per cent. and 95 per cent. inhibition of the response to $2k$). Part explanation for this tendency may be deduced from Figure 4. Here, fiducial limits ($P = 0{\cdot}95$), expressed as $\pm$ percentages of the mean, have been plotted as ordinates against the percentage range of the $2k$ response employed in each experiment. When widely different degrees of inhibition were produced by the three selected doses of inhibitor, the experiment covered a large percentage range of the uninhibited response to $2k$. Figure 4 indicates that estimates from wide range experiments in this series tend to be more reliable than those from narrow range experiments.

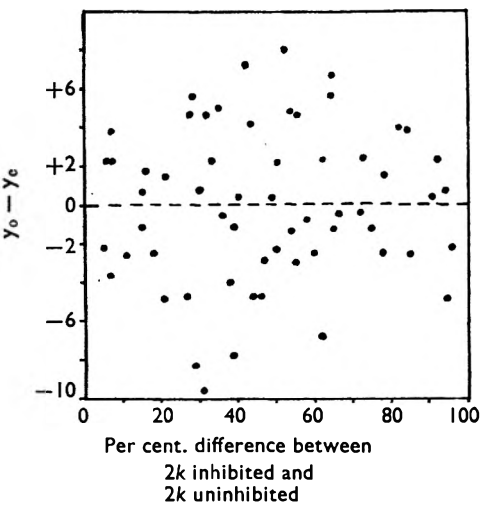

FIG. 3. Investigation of the incidence of deviation from linearity in the regression of y on x during determinations of pA_2 at 2 min. See text for explanation. Ordinates: differences between observed (y) and calculated (y_c) values of y. Abscissæ: percentage differences between responses to 2 k of activating drug, before and after antihistamine.

The fact that the fiducial limits ($P = 0{\cdot}95$) encountered in this series of experiments with crude extracts were wider than those recorded when pure drugs were used (cf. Figure 4 and Table IV) is attributed to the presence of other substances with weak pharmacological actions in these extracts together with the antihistamine.

EXAMINATION OF THE RESULTS OBTAINED BY THE METHOD ADVOCATED

Comparison has been made in Table IV of values of pA_2 at 2 minutes obtained for mepyramine-histamine, diphenhydramine-histamine, and atropine-acetylcholine by

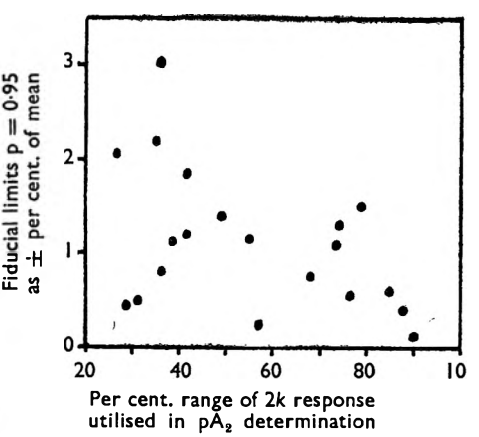

FIG. 4. Calculated fiducial limits related to the range of the response to $2k$ used during pA_2 determinations. Ordinates: fiducial limits ($P = 0{\cdot}095$) expressed as $\pm$ per cent. mean. Abscissæ: per cent. range of the response to $2k$ utilised during the determination. See text for explanation.

Schild[7] in column 1 and by the new method in column 2. In each case the mean value has been entered ± the standard error of the mean followed by the number of experiments within brackets. There is good agreement between the two sets of results. Six values for pA_2 at 2 minutes for atropine-acetylcholine on rat ileum are included in column 2, since these values have not, in our experiments, differed from those obtained using guinea-pig ileum. All other values in this table were obtained with guinea-pig ileum.

TABLE IV

SUMMARY OF pA_2 VALUES OBTAINED, AND OF CALCULATED LIMITS OF ERROR

pA_2 values ± S.E. (N)		Fiducial limits of error (P = 0·95) for method described	
Schild[7] (1)	Method described (2)	In individual experiments (3)	Directly estimated (4)
Mepyramine-histamine 8·71 ± 0·021 (4)	8·711 ± 0·018 (8)	± 0·22 to 2·10 per cent.	± 0·84 per cent.
Diphenhydramine-histamine 7·75 ± 0·035 (8)	7·735 ± 0·090 (6)	± 0·21 to 2·11 per cent.	± 1·46 per cent.
Atropine-acetylcholine 8·27 ± 0·021 (11)	8·246 ± 0·026 (11)	± 0·41 to 2·80 per cent.	± 2·51 per cent.

The error of each estimate of pA_2 at 2 minutes was calculated from the internal evidence of the test; fiducial limits (P = 0·95) were applied and were expressed as ± percentages of the means. The ranges of these limiting values were entered in Table IV, column 3. Direct estimates of the error of the method were made by expressing the difference of each calculated value of pA_2 from its mean as a percentage of that mean, squaring and summing the percentage differences, then dividing by N-1 to obtain the variance in the usual way. The square roots of the variances multiplied by the value of t(P = 0·95) for N-1 degrees of freedom gave the direct estimates of the fiducial limits entered in Table IV, column 5. For each pair of drugs the fiducial limits obtained by direct estimation fell within the range of values calculated from the internal evidence of individual experiments. The small numbers of observations used for the direct estimates of error are undoubtedly largely responsible for this finding. It may, however, be concluded that the fiducial limits obtained for individual estimates of pA_2 are satisfactorily wide.

SUMMARY

1. A method for the determination of pA_2 at 2 minutes has been presented.

2. The method allows the calculation of limits of error from the internal data of the experiment.

3. Fiducial limits of error (P = 0·95) obtained for mepyramine-histamine, diphenhydramine-histamine, and atropine-acetylcholine were always less than ± 3·0 per cent. of the mean.

4. Two such estimates can regularly be made by a skilled worker in seven hours, using only a single, manually operated, isolated organ bath.

This work was carried out whilst A. L. Bartlet was in receipt of a D.S.I.R. award.

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A FURTHER NOTE ON THE GRAVIMETRIC DETERMINATION OF SANTONIN

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Artemisia is an important commercial drug. Several species of Artemisia contain santonin, which is a reliable specific for the elimination of intestinal roundworms. The important santonin-containing species of commercial value are, (i) *Artemisia Cina* Berg., (ii) *Artemisia kurramensis* Qazilbash, (iii) *Artemisia brevifolia* Wall, (iv) *Artemisia maritima* L., and (v) *Artemisia pauciflora* Weber.

In West Pakistan, *Artemisia kurramensis* Qazilbash and *Artemisia brevifolia* Wall, grow abundantly in commercial quantities in the Kurram and Gilgit Agencies respectively. Pakistan artemisias possess a great economic value in the world drug market on account of their high santonin-content.

The commercial value of artemisia is determined on the basis of its santonin-content. The assay of artemisia, therefore, is of considerable importance in assessing the market value of the drug. The writer has devoted much time and attention to the economic study of different species of Artemisia, growing in West Pakistan, Afghanistan and parts of Iran. A very large number of samples were collected, from time to time, during a period of about 30 years, from different geographical areas. The samples were thoroughly examined and assayed for their santonin-contents.

The amount of santonin in proportion to associated oily and resinous constituents is variable in different species of Artemisia. The relative proportion also varies in plants of the same species, growing in different ecological conditions.

The Assay of Santonin

As a result of extensive field tests at different altitudes in different geographical regions, and intensive laboratory work at Peshawar, in connection with the commercial utilisation of the Kurram and Kashmir artemisias, some slight modifications have been made in the author's assay method[1,2]. The important consideration in developing the technique, was to establish an economical, simple and reliable method of general application. The important modifications lately adopted are noted below: (i) the dried benzol extract is heated with the barium hydroxide solution on a steam bath, (ii) a stoppered Erlenmyer flask is used in place of a crystallising dish, (iii) the volume of the final filtrate from which the crystals of refined santonin, have been removed, is determined; and a correction-factor is added on the basis of 6·4 mg. per 10 ml. of the filtrate, to the weight of santonin finally obtained. The correction-factor takes into account the solubility of santonin in the ethanol solution

as well as the adsorption of santonin by the mixture of animal charcoal and kieselguhr.

Kassner *et al.*[3] have criticised the author's method[1] and have proposed an assay method, partly derived from the author's method and partly based on the assay method in use in their laboratory. They have adopted several modifications which may be summarised: (i) The use of bruised drug in place of the powdered material, (ii) percolation to exhaustion with the solvent, (iii) the use of saturated solution of barium hydroxide in place of 5 per cent. freshly prepared solution of barium hydroxide, (iv) the use of chloroform to dissolve the dried benzol extract before treating it with a saturated solution of barium hydroxide, and (v) the use of animal charcoal in place of a mixture of animal charcoal and kieselguhr.

The following comments are offered on the various points.

(i) *Use of the bruised drug in place of the powdered material.* Bruised material of *Artemisia brevifolia* Wall requires a greater volume of benzene than the powdered material to produce the required aliquot portion. When powdered material is used, the penetration of the alkali is more rapid and satisfactory and the subsequent extraction with benzene is adequate.

(ii) *Percolation to exhaustion.* Percolation to exhaustion requires a larger quantity of benzene than taking an aliquot portion after proper maceration which is more economical and gives satisfactory results. The writer has been concerned mainly with collections from remote hilly tracts, where transport is difficult and very expensive. An appreciable quantity of ballast matter is also carried away in the procedure, when the material is completely exhausted by large amount of the solvent. Maceration and taking an aliquot portion gives less trouble and the final yield is better.

(iii) *Use of a greater volume of saturated solution of barium hydroxide in place of 5 per cent. freshly prepared solution of barium hydroxide.* Kassner *et al.* consider 100 ml. of freshly prepared 5 per cent. solution of barium hydroxide insufficient for extracting all the available santonin from the benzol extract. They have suggested the use of a relatively greater volume of the saturated solution of barium hydroxide. Careful stirring with a glass rod brings about complete conversion of all the available santonin, when 110 ml. of freshly prepared 5 per cent. solution of barium hydroxide is used. The excess of barium hydroxide is undesirable. Theoretical calculations in regard to the chemical reactions involved do not justify the use of a greater volume of barium hydroxide solution, as suggested by them.

(iv) *Use of chloroform in dissolving the dried benzol extract.* When the dried benzol extract is dissolved in chloroform, and the resulting chloroformic extract is treated with a saturated solution of barium hydroxide as suggested by Kassner *et al.*, then the purity of the final yield of santonin suffers on this account. Better yield is obtained when the dried benzol extract is directly treated with the barium hydroxide solution.

(v) *Use of animal charcoal in place of a mixture of animal charcoal and kieselguhr.* Kassner *et al.* are of opinion that "kieselguhr is unnecessary

and only hinders filtration." Kieselguhr is universally known as filter-aid. Under normal weather conditions no hindrance in filtration is offered, when a mixture of equal parts of kieselguhr of suitable texture,

TABLE I

YIELDS OF SANTONIN AFTER USING KIESELGUHR AND CHARCOAL, OR CHARCOAL ALONE FOR CLARIFICATION

Artemisia species	Locality	Date of collection	Santonin per cent.	Melting point °C.	Composition of clarifying agent used
Artemisia kurramensis	Shublan	8.3.52	1·67	172.0	Mixture consisting of 2 parts kieselguhr and 3 parts animal charcoal
,, ,,	,,	,,	1·68	172·1	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
Artemisia brevifolia	Rattu	15.8.50	1·37	173.0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	1·25	172·2	Mixture consisting of 2 parts kieselguhr and 3 parts animal charcoal
,, ,,	Rampur	,,	1·15	172·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	1·14	172·0	Mixture consisting of 2 parts kieselguhr and 3 parts animal charcoal
Artemisia kurramensis	Parachinar	20.9.53	1·75	170·6	Animal charcoal alone
,, ,,	,,	,,	1·85	172·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	Zairan	22.9.53	1·41	171·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	4·38	171·0	Animal charcoal alone
,, ,,	Paiwar	,,	1·27	172·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	1·23	172·0	Animal charcoal alone
,, ,,	Alizai	,,	1·42	171·3	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	1·38	171·0	Animal charcoal alone
,, ,,	Islamia College	25.7.53	0·85	171·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	0·84	170·0	Animal charcoal alone
,, ,,	,,	22.9.53	1·29	173·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	0·98	173·2	Animal charcoal alone
,, ,,	Nastikote	20.9.53	1·73	168·2	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	1·69	167·0	Animal charcoal alone
,, ,,	Islamia College	14.7.53	1·18	174·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	1·15	173·2	Animal charcoal alone
,, ,,	,,	20.7.53	0·84	173·0	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal
,, ,,	,,	,,	0·84	172·0	Animal charcoal alone
,, ,,	,,	25.7.53	0·84	170·0	Animal charcoal alone
,, ,,	,,	,,	0·85	170·4	Mixture consisting of 1 part kieselguhr and 1 part animal charcoal

and finely powdered animal charcoal is employed. In cold weather, when the temperature is low, a steam jacket could be used with advantage for rapid, efficient filtration. Kieselguhr is a good adsorbent, and its use is helpful in removing the colloidal resinous impurities, which otherwise escape removal.

Experimental evidence. Under controlled experimental conditions, samples of different species of Artemisia were assayed and the following reagents were employed as clarifying agents for the removal of impurities: (i) a mixture of 2 parts of kieselguhr and 3 parts of animal charcoal, (ii) a mixture of equal parts of kieselguhr and animal charcoal, (iii) animal charcoal alone.

The best results were obtained when a mixture of equal parts of kieselguhr and animal charcoal was used. When animal charcoal alone was employed, there was a greater loss of recoverable santonin, and there was noticeable deterioration in the final yield of santonin, as judged by colour and melting point. The results are given in Table I.

Experiments have also been made on the adsorption of santonin by (i) a mixture of equal parts of animal charcoal and kieselguhr and (ii) animal charcoal alone; under similar experimental conditions. The details are given below.

(i) *A mixture of equal parts of animal charcoal and kieselguhr.* A known quantity of pure santonin was boiled with 50 ml. of 15 per cent. ethanol w/w, under a reflux for 15 minutes, and filtered hot into an Erlenmeyer flask. The flask and the filter paper were washed three times with 5 ml. warm 15 per cent. ethanol. The filtrate was heated with 100 mg. of a mixture of equal parts of finely powdered animal charcoal and kieselguhr, under a reflux condenser for 10 minutes and filtered hot into a crystallising dish. The residue and the filter-paper were thrice rinsed with 5 ml. of the 15 per cent. ethanol. The crystallising dish was kept in the dark at 15°–17° C. for 24 hours. The crystals of santonin were collected on a weighed filter-paper (preheated at 103°–105° C. and cooled in a dessicator), and the crystallising and the filter-paper washed twice with 5 ml. of the 15 per cent. ethanol. The volume of the filtrate was determined. The crystals of santonin were dried at 103° to 105° C. and placed in a dessicator over sulphuric acid. The weight of santonin was then determined. The results are given below in Table II.

TABLE II

YIELD OF SANTONIN AFTER CLARIFICATION WITH EQUAL PARTS OF CHARCOAL AND KIESELGUHR

Weight of santonin taken (mg.)	Weight of santonin regained (mg.)	Loss in weight (mg.)	Volume of final filtrate (ml.)	Correction-factor per 10 ml. of the final filtrate
203·3	160·7	42·6	64·1	6·6
201·7	160·7	41·0	65·3	6·3
200·0	156·4	43·6	66·5	6·5
100·0	57·4	42·6	73·1	5·8
100·0	58·0	42·0	72·3	5·8

It was realised that the volume of the final ethanolic solution containing the refined santonin crystals, is noticeably influenced by weather conditions such as temperature, atmospheric humidity, and the movements of air-currents. In view of such considerations, a stoppered Erlenmeyer flask was substituted in place of the crystallising dish in later work. Two sets of experiments were carried out. In one case 100 mg. of a mixture

of equal parts of kieselguhr and animal charcoal was used and in the other case 100 mg. of animal charcoal alone was employed. The results are shown in Table III and Table IV respectively.

TABLE III

EXPERIMENT (A): A MIXTURE OF EQUAL PARTS OF KIESELGUHR AND ANIMAL CHARCOAL USED

Weight of santonin taken (mg.)	Weight of santonin regained (mg.)	Volume of the final filtrate (ml.)	Total loss in weight (mg.)	Loss in weight due to solubility in the filtrate	Other loss due to adsorption by the mixture of kieselguhr and animal charcoal
100	55·4	83	44·6	26·6	18·0
100	55·2	83	44·8	26·6	18·2
100	54·9	83	45·1	26·6	18·5
100	57·4	84	42·6	26·9	15·7
150	103·8	84	48·7	27·2	21·5
150	102·1	85	46·2	26·9	19·3
150	102·8	84	47·9	27·2	20·7
150	101·3	85	47·2	26·9	20·3
200	143·7	84	56·3	26·9	29·4
200	143·0	84	57·0	26·9	30·1
200	143·9	83	56·1	26·6	29·5
200	145·2	84	54·8	26·9	27·9

TABLE IV

EXPERIMENT (B): ANIMAL CHARCOAL ALONE USED

Weight of santonin taken (mg.)	Weight of santonin regained (mg.)	Volume of the final filtrate (ml.)	Total loss in weight (mg.)	Loss in weight due to solubility in the filtrate (mg.)	Other loss due to adsorption by animal charcoal (mg.)
100	34·8	84·0	65·2	32·8	32·4
100	38·0	83·0	62·0	32·4	29·6
150	87·8	83·0	62·2	32·4	29·8
150	85·5	83·5	64·5	32·6	31·9
200	129·5	83·5	70·5	32·6	37·9
200	131·3	83·0	68·7	32·4	36·3

Reference to Tables II, III and IV shows that there is a greater loss of santonin, when animal charcoal alone is used than when a mixture of kieselguhr and animal charcoal is employed. It is interesting to note that the adsorption of santonin is variable and inconsistent. The adsorption of colloidal resinous substances as well as santonin is influenced by the purity and degree of fineness of kieselguhr and animal charcoal. Due regard should therefore be paid to the standard quality and texture of the material used.

Badhwar *et al.*[4] have also shown that santonin adsorbed by animal charcoal is neither negligible nor consistent. They have recommended that treatment with animal charcoal be altogether avoided.

CONCLUSION

The assay method lately developed by Kassner *et al.*[3] is lengthy, time consuming, and requires large quantities of the solvent and reagents. The revised method proposed here is more economical and less time consuming; and the results are better qualitatively as well as quantitatively. The

final yield is white and has a melting point of 171° to 174° C. The method is also of general application. Also, with suitable adjustments, the method could be adopted for the isolation of santonin on an industrial scale. As a measure of normal routine the method gives the best results with good quality artemisias. Artemisias of low santonin-content deserve no serious consideration for purposes of commercial utilisation. Material totally devoid of santonin, or of poor quality, can easily be sorted out with the help of the potassium methoxide test. Experience with colour indications readily gives a clear idea of the approximate amount of santonin present. The chief interest of the author has been to arrange commercial collections of artemisia with high santonin-content from selected areas. The method could, however, be used for the quantitative determination of santonin in poor quality artemisias, by adopting slight modifications in the quantitative details. For this purpose, a larger quantity of the drug is taken, and suitable adjustments are made accordingly. At the final stage, the dried chloroformic extract is boiled with 50 ml. of ethanol (15 per cent. w/w), and proceeded with in the usual way. The amount of santonin could easily be estimated.

If enough artemisia is not in hand and only a limited quantity is available for assay purposes, the best alternative would be to mix the artemisia of poor quality with an equal quantity of best quality artemisia of known santonin-content (about 2 per cent.), and determine the amount of santonin in the mixture. The percentage of santonin in poor quality artemisia could be calculated.

Summary

1. Some modifications have been made to the author's method of assay of artemisia reported in 1951.

2. Comments and experimental observations, relative to the various points raised by Kassner *et al.*,[3] are submitted.

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A NOTE ON THE PHARMACOLOGY OF PHENOXYMETHYL-PENICILLIN (PENICILLIN V)

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Oral administration, which is a therapeutic advantage, was not always justified with benzylpenicillin owing to its destruction in the acid medium of the stomach[1,2] and by penicillinase in the ileum[3]. Attempts to overcome these difficulties by the use of antacids and penicillinase inhibitors were not wholly successful in practice. The introduction of the long-acting insoluble penicillins, namely procaine and benzathine penicillin, although solving the problem of frequent intramuscular injections did not lead to a great advance in oral penicillin therapy. Oral procaine penicillin combined with sodium benzoate is reported as a satisfactory form of oral penicillin[4]. The results with benzathine penicillin (*NN'*-dibenzylethylenediamine dipenicillin), after oral administration were contradictory; some reported satisfactory serum concentrations after its use by mouth[5–8], while others did not obtain satisfactory results[9–11].

Phenoxymethylpenicillin, or Penicillin V is another form of insoluble penicillin. It was prepared biosynthetically by Behrens and Kingkade[12]. Brandl and Margreiter[2], demonstrated that it is insoluble in acid but soluble in alkaline medium and hence its antibacterial power is not appreciably reduced in the stomach. They showed that after two hours' exposure to hydrochloric acid at pH 2·8, the loss of potency of phenoxymethylpenicillin compared with benzylpenicillin is 9·2 and 44·4 per cent. respectively. A preliminary report[13] shows that it is rapidly absorbed from the duodenum producing adequate serum concentrations for 4 hours after a dose of 100,000 units. In the present study the concentration of penicillin in the serum and in the urine of normal adults was estimated after oral administration of different doses of phenoxymethylpenicillin.

Material and Methods

Phenoxymethylpenicillin was given to normal adult medical students as well as to members of the staff of this department. Each of a group of 10 volunteers was given a single dose of 600,000 units (6 tablets), of phenoxymethylpenicillin, on an empty stomach. The products were swallowed intact, to avoid prolonged contact with the saliva. Samples of blood were taken from one of the cubital veins at intervals of 1, 2, 3, 4, 5 and 6 hours, three samples being taken from each subject alternately. The blood samples were centrifuged to separate the serum which was used for the assay. To each member of a second group of 20 volunteers, a single dose of 100,000 units each (one tablet of 60 mg.) was given on an empty stomach as above. Samples of blood were taken at intervals of ½, 1, 2, 4 and 6 hours. Urine samples were also taken from the latter group who

were made to empty the bladder completely before swallowing the tablets. The urine was collected at ½, 1, 2, 4, 6, 8 and 12 hours intervals. The urine was filtered and sterilised by heating at 60° C. for half an hour, to kill interfering bacteria[14] and then stored in the refrigerator until required.

The technique adopted for penicillin assay was the agar plate diffusion method using *Staph. aureus* (Oxford strain) as a test organism. The standard used for comparison was a stock solution of benzylpenicillin prepared fresh weekly and kept in a refrigerator. From this stock solution, suitable dilutions were prepared at the time of the assay. A duplicate assay was carried out on each sample of urine or blood. After incubation overnight, the diameter of the inhibition zones was read for the two plates and averaged. The concentration of penicillin in the unknown sample was read from a curve drawn for the standard concentrations.

Results

Table I shows the penicillin serum concentration after a single dose of 600,000 units. From the Table it is seen that there is a high penicillin concentration one hour after administration to the fasting stomach. In only one of five was this concentration very low. During the following 3 hours, penicillin concentration declined slowly to an average of 0·15 units/ml. at the end of the fourth hour. It will be noted that individual variations are considerable. At the end of 6 hours the serum of all 4 volunteers tested still showed an inhibitory penicillin concentration, though to a minimum degree. The comparatively high penicillin concentration at the end of 4 hours, together with the suggestion of the preliminary report led us to the use of a smaller oral dose.

TABLE I

PENICILLIN SERUM CONCENTRATION IN NORMAL ADULTS AFTER A SINGLE ORAL DOSE OF PHENOXYMETHYLPENICILLIN

(DOSE: 600,000 UNITS APPROX.)

	Penicillin concentration in units/ml.					
Number	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.
1	0·25	—	0·61	—	—	—
2	—	0·46	—	0·35	—	—
3	0·03	—	—	—	0·03	—
4	—	0·03	—	0·14	—	0·03
5	0·71	—	0·10	—	0·06	—
6	—	0·24	—	0·06	—	0·03
7	0·19	—	0·36	—	—	—
8	—	0·44	—	0·04	—	0·04
9	1·19	—	0·45	—	—	—
10	—	0·34	—	0·16	—	0·03
Mean	0·46	0·30	0·38	0·15	0·04	0·03
S.D.	0·47	0·18	0·21	0·12	0·03	0·005

— = No sample taken.

Table II shows the penicillin serum concentration after a single dose of 100,000 units to a fasting stomach. In this case of 9 samples taken half an hour after administration, 6 showed an inhibitory concentration of penicillin, but in 3 no such concentration could be detected. As before, individual variation is great. It was found that the mean penicillin concentration rises rather rapidly during the first hour to 0·19 units/ml. and then declines during the next 3 hours. At the end of 4 hours 6 out of 15 persons showed an inhibitory penicillin concentration in the serum. At the end of 6 hours there was practically no penicillin in the blood.

Comparison of the results recorded in Tables I and II show that although the one dose is 6 times larger than the other, the penicillin serum concentration is just over twice as much; this is a well-known pharmacological

phenomenon. It will be also seen that a dose of 100,000 units (one tablet) maintains an effective serum concentration adequate for most susceptible infections for a duration of 4 hours. The reduction of penicillin concentration at the end of 4 hours to the mean value of 0·02 units/ml. does not mean that the antibiotic effect has ceased altogether because it is known that penicillin will be found in the tissues for some time after its disappearance from the blood.

Excretion in the urine. Table III shows that penicillin has been detected in the urine in all our cases half an hour after oral administration with an average concentration of 5·6 units/ml. Excretion continued and the antibiotic is present in the urine in a concentration 1000 times or more its blood level. The maximum urinary concentration was obtained 2 hours after administration and at 12 hours, a considerable amount (1·3 units/ml.) was still present.

Side effects. In this series side effects were noticed in two subjects in the first group (6 tablets); one complained of a sensation of heaviness in

TABLE II

PENICILLIN SERUM CONCENTRATION IN NORMAL ADULTS AFTER A SINGLE ORAL DOSE OF PHENOXYMETHYLPENICILLIN

(DOSE: 100,000 UNITS APPROX.)

	Penicillin concentration in units/ml.				
Number	½ hr.	1 hr.	2 hr.	4 hr.	6 hr.
1	0·09	—	0·12	—	—
2	—	0·03	—	0·03	0·39
3	—	—	0·03	N.I.	—
4	0·19	—	0·21	N.I.	—
5	—	0·30	—	N.I.	N.I.
6	0·12	—	0·27	N.I.	N.I.
7	0·29	—	0·11	N.I.	—
8	—	0·69	—	—	N.I.
9	—	0·03	—	—	—
10	—	0·21	—	N.I.	N.I.
11	0·03	—	0·07	0·08	—
12	N.I.	—	0·23	N.I.	—
13	—	0·04	—	—	N.I.
14	N.I.	—	—	0·14	—
15	—	0·13	0·07	—	N.I.
16	—	0·21	—	N.I.	N.I.
17	—	0·20	—	0·03	N.I.
18	0·06	—	0·17	N.I.	—
19	—	0·07	0·08	0·06	—
20	N.I.	—	0·03	0·03	—
Mean	0·09	0·19	0·13	0·02	
S.D.	0·08	0·197	0·08	0·04	

— = No sample taken.
N.I. = Non-inhibitory concentration.

TABLE III

PENICILLIN CONCENTRATION IN URINE OF NORMAL ADULTS AFTER A SINGLE ORAL DOSE OF PHENOXYMETHYLPENICILLIN

(DOSE: 100,000 UNITS)

	Penicillin concentration in units/ml.						
Number	½ hr.	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.	12 hr.
1	1·70	—	85·60	25·00	—	—	—
2	19·00	—	400·00	57·50	—	0·60	N.I.
3	—	44·00	—	—	—	10·00	N.I.
4	5·00	—	1187·50	362·50	58·75	—	—
5	14·00	—	775·00	155·65	—	—	7·50
6	—	830·00	—	—	4·40	—	—
7	3·10	—	850·00	—	147·50	1·36	0·36
8	—	303·00	—	—	—	1·09	0·29
9	1·63	—	8·75	194·00	—	—	—
10	—	0·61	—	—	50·00	—	0·60
11	—	1·00	50·00	1125·00	—	—	0·50
12	—	625·00	1700·00	581·50	44·00	—	4·40
13	7·13	—	212·50	587·50	27·50	—	—
14	0·64	—	156·50	13·75	—	—	0·25
15	—	6·38	—	—	77·50	—	—
16	3·44	—	—	350·00	—	—	0·13
17	—	53·75	58·75	—	31·30	—	—
18	—	118·75	—	75·00	—	—	—
19	0·06	—	53·75	53·75	2·00	—	—
20	—	4·50	52·50	197·00	—	—	0·07
Mean	5·60	198·70	430·10	290·60	49·20	3·30	1·30
S.D.	6·24	297·30	537·60	318·50	44·20	4·50	2·35

— = No sample taken. N.I. = Non-inhibitory concentration.

the stomach and the other of joint pains especially in the wrist and fingers. These symptoms disappeared next morning.

SUMMARY

1. Penicillin serum concentrations were estimated on several occasions after a dose of 600,000 units of Penicillin V given once to each of 10 persons and after a dose of 100,000 units of Penicillin V given once to each of 20 persons. Serum concentrations from $\frac{1}{2}$ to 6 hours are tabulated.

2. Penicillin concentration in the urine was estimated in the latter group and very high levels for 12 hours were obtained. Side effects were slight.

3. It is suggested that this new penicillin may prove effective for oral penicillin therapy.

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ESTIMATION OF CAMPHOR IN PHARMACEUTICAL PREPARATIONS

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Various methods for the estimation of camphor and camphor containing preparations, both colorimetric[1,2] and volumetric[3,4,5,6,7,8,9] have been proposed, but no method is known which is uniformly applicable to all the pharmaceutical preparations containing camphor. Castiglioni[1] has suggested that when camphor is heated in ethanolic solution with furfurol or benzaldehyde and sulphuric acid, a violet colour or bright red colour respectively, is produced which can be used for the determination of the amount of camphor present. This method may be applicable to camphor, and spirit of camphor but in liniment of turpentine the oil of turpentine interferes with the colour production. The same is true of the method advocated for camphor by Mitchell[2], wherein when camphor is heated with 2:4-dinitrophenylhydrazine reagent and hydrochloric acid and cooled, a wine-red colour is produced on the addition of ethanolic solution of potassium hydroxide.

The chemical methods are similar to those employed for the estimation of carbonyl compounds using 2:4-dinitrophenylhydrazine or hydroxylamine hydrochloride reagent. The method with the former reagent is mostly gravimetric[3,4,5] while the latter reagent can be used for both gravimetric[6] as well as volumetric[5,7] estimations.

In the B.P. 1953 method[3] the 2:4-dinitrophenylhydrazine reagent is refluxed with camphor and the 2:4-dinitrophenylhydrazone which is formed is weighed. This method gives results which are too low[5] owing to the decomposition of dinitrophenylhydrazone by heat, during refluxing. In the N.F. IX method[8] the over-heating is avoided by using a pressure-heating method, which is tedious and risky, though Ozger[5] finds the results to be more accurate.

In the present investigation, when the B.P. 1953 method[3] was used to estimate camphor in liniment of turpentine, the precipitate of 2:4-dinitrophenylhydrazone could not be separated since it remained dissolved in turpentine-oil. Attempts to remove the turpentine oil by steam-distillation resulted in a sticky mass which could not be properly washed and dried. Besides the above method, Goldstein and Reindollar[9] have suggested a method for the estimation of camphor, on the lines recommended by the N.F. IX[10], for the assay of spearmint spirit. In this method, the volume of the separated oily layer is read after the addition of acid calcium chloride solution and a known volume of kerosene. But this method is not suitable for liniment of turpentine which contains turpentine oil in addition to camphor.

In the present investigations, successful attempts have been made to estimate camphor volumetrically in pharmaceutical preparations on the

lines suggested by Ozger[5] and Wolstadt[7], using hydroxylamine hydrochloride. The camphor or its preparation is refluxed with hydroxylamine hydrochloride reagent in ethanol 90 per cent. for 4 hours in the presence of sodium bicarbonate. The reaction mixture is cooled and the condenser is washed with 20 ml. of light petroleum (boiling point 50° to 60°) which also aids a sharp end-point during titration. The mixture is then titrated using dimethyl yellow as indicator, with standard hydrochloric acid which neutralises the excess of sodium bicarbonate and converts the unreacted hydroxylamine to hydroxylamine hydrochloride. The solution is then titrated with standard potassium hydroxide solution using phenolphthalein as an indicator, which determined the unreacted hydroxylamine hydrochloride. A simultaneous blank experiment is conducted and the difference in the titrations between the blank and the sample gives the amount of hydroxylamine hydrochloride reacted with camphor which is calculated from the factor given in the experimental part.

In order to test the suitability of this method for camphor-containing pharmaceutical preparations, a 5 per cent. solution of camphor was made in steam distilled turpentine oil and the camphor estimated by the above method. The analysis of other pharmaceutical preparations was made after obtaining satisfactory results in this estimation.

Experimental

Reagents

Ethanol 95 per cent. (aldehyde free). 2·5 g. of lead acetate was dissolved in 5 ml. of water and the solution was added to 100 ml. of ethanol 95 per cent. contained in a glass stoppered bottle. The whole mixture was shaken thoroughly. 5 g. of potassium hydroxide was dissolved in 25 ml. of warm ethanol. The solution was cooled and added slowly to the ethanolic solution of lead acetate. After one hour, the mixture was shaken thoroughly and allowed to stand overnight. The ethanol was recovered by distillation.

Hydroxylamine hydrochloride solution. 4 g. of hydroxylamine hydrochloride was dissolved in ethanol 90 per cent. (aldehyde free) to make 100 ml.

Sodium Bicarbonate of B.P. quality.

Turpentine oil, steam distilled. 26·5 g. of Sodium Hydroxide B.P. was dissolved in 500 ml. of distilled water. This solution was mixed with 500 ml. of turpentine oil and about three-fourths of the oil collected by steam distillation. The oily portion was separated from the water, dried by shaking with anhydrous calcium chloride and filtered.

Estimation of Camphor

Camphor in oil of turpentine. 5 g. of camphor accurately weighed was dissolved in oil of turpentine (steam-distilled) to make 100 ml. 5 ml. of this camphor solution was refluxed for 4 hours with 20 ml. of ethanol 95 per cent. (aldehyde free), 10 ml. of hydroxylamine hydrochloride reagent and 0·3 g. of sodium bicarbonate, cooled and the condenser was

rinsed with 20 ml. of light petroleum (boiling point 50° to 60° C.) into the flask. The mixture was titrated with 0·2N hydrochloric acid using dimethyl yellow as an indicator. The control was also adjusted to the same tint. Both solutions were then titrated to pink colour with 0·2N potassium hydroxide solution using phenolphthalein as an indicator. The amount of camphor was calculated from the difference in the volume of 0·2N potassium hydroxide used in the two titrations. Each ml. of 0·2N potassium hydroxide is equivalent to 0·0304 g. of camphor. (In the control experiment 5 ml. of steam distilled turpentine oil was added before refluxing.) (Table I.)

TABLE I

RECOVERY OF CAMPHOR FROM OIL OF TURPENTINE

Experimental per cent. of camphor w/v	Theoretical per cent. w/v
4·92	5·00
4·89	5·00
4·98	5·00

Camphor

The assay was carried out as described under camphor in turpentine oil, using 0·2 g. of camphor in place of 5 ml. of camphor in oil solution. (Table II.)

TABLE II

RECOVERY OF CAMPHOR

Amount of camphor taken	Experimental per cent. of camphor w/w	B.P. Standard
0·279 g.	98·07	Not less than 96 per cent.
0·3207 g.	99·00	do.
0·177 g.	98·00	do.

Spirit of Camphor

The assay was carried out as described under camphor in turpentine oil, using 2 ml. of spirit of camphor in place of 5 ml. of camphor in oil solution. (Table III.)

Liniment of Camphor

About 1 g. of liniment of camphor, accurately weighed, was refluxed with 30 ml. of ethanol, 95 per cent. (aldehyde free), 10 ml. of hydroxylamine hydrochloride reagent and 0·3 g. of sodium bicarbonate. The assay was completed as described under camphor in turpentine oil commencing at the words, "Cooled and the condenser was rinsed with 20 ml. of light petroleum into the flask. . . ." (In the control experiment 1 g. of arachis oil was added before refluxing.) (Table IV.)

TABLE III

RECOVERY OF CAMPHOR FROM SPIRIT OF CAMPHOR

Experimental per cent. of camphor w/v	Theoretical per cent. w/v
10·03	10·00
9·88	10·00
9·95	10·00

Liniment of Turpentine

About 50 g. of liniment of turpentine, accurately weighed, was diluted with 50 ml. of distilled water, cooled and acidified with dilute sulphuric acid, using solution of methyl orange as indicator. The mixture was distilled in steam and the distillate was collected in a separator, till all the volatile matter was distilled. The aqueous portion of the distillate was separated out and the oily layer was transferred to a 250 ml. measuring

flask. The condenser and the separator were rinsed each with 50 ml. of ethanol 95 per cent. (aldehyde free) into the same measuring flask. The aqueous portion was extracted with 30 ml. of solvent ether and the ethereal extract was transferred into the measuring flask. The volume was adjusted to 250 ml. with sufficient quantity of ethanol 95 per cent. (aldehyde free). 20 ml. of this solution was refluxed with 10 ml. of hydroxylamine hydrochloride solution and 0·3 g. of sodium bicarbonate. The assay was completed as described under camphor in turpentine oil, commencing at the words, "cooled and the condenser was rinsed with 20 ml. of light petroleum into the flask. . . ." (In the control experiment, 2·5 ml. of steam-distilled turpentine oil was added before refluxing.) (Table V.)

TABLE IV

RECOVERY OF CAMPHOR FROM LINIMENT OF CAMPHOR

Amount of preparation taken	Experimental per cent. of camphor w/w	Theoretical per cent. w/w
1·085 g.	19·2	20·0
1·285 g.	19·6	20·0
1·026 g.	19·7	20·0

TABLE V

RECO ERY OF CAMPHOR FROM LINIMENT OF TURPENTINE

Amount of preparation taken	Experimental per cent. of camphor w/v	Theoretical per cent. w/v
44·695 g.	4·95	5·0
45·857 g.	4·90	5·0
45·453 g.	4·92	5·0

Ammoniated Liniment of Camphor

20 ml. of ammoniated liniment of camphor was mixed with 30 ml. of turpentine oil steam distilled and the mixture diluted with 50 ml. of distilled water. The assay was completed as described under Liniment of Turpentine, commencing at the words, "acidified with dilute sulphuric acid, using solution of methyl orange. . . ." (In the control experiment, 2·5 ml. of steam distilled turpentine oil was added.) (Table VI.)

TABLE VI

RECOVERY OF CAMPHOR FROM AMMONIATED LINIMENT OF CAMPHOR

Experimental per cent. of camphor w/v	Theoretical per cent. w/v
12·26	12·5
12·16	12·5
12·35	12·5

TABLE VII

RECOVERY OF CAMPHOR FROM LINIMENT OF SOAP

Experimental per cent. of camphor w/v	Theoretical per cent. w/v
3·88	4·0
3·87	4·0
3·95	4·0

Liniment of Soap

50 ml. of liniment of soap was mixed with 30 ml. of turpentine oil (steam distilled) and the mixture diluted with 50 ml. of distilled water. The assay was completed as described under Liniment of Turpentine, commencing at the words, "acidified with dilute sulphuric acid, using solution of methyl orange. . . ." (In the control experiment, 2·5 ml. of turpentine oil, steam distilled and 0·06 ml. of oil of rosemary were added.) (Table VII.)

Summary

1. A volumetric method has been described for the estimation of camphor in various pharmaceutical preparations. This consists of reacting the preparation with an ethanolic solution of hydroxylamine hydrochloride in the presence of sodium bicarbonate and titrating the unreacted hydroxylamine hydrochloride with 0·2N hydrochloric acid and 0·2N potassium hydroxide, using dimethyl yellow and phenolphthalein respectively as indicators. In all the estimations a control experiment is necessary.

2. All seven estimations by this method give results within 96 per cent. to 102 per cent.

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OXIDISED CELLULOSE IN ION EXCHANGE
A PRELIMINARY NOTE

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The product, resulting from the oxidation of a large portion of the primary hydroxyl groups of cellulose to carboxyl, by nitrogen dioxide, is a polyanhydroglucuronic acid referred to as oxidised cellulose. In physical appearance oxidised cellulose differs little from ordinary cellulose. It will, however, dissolve readily in alkaline solutions such as sodium or ammonium hydroxides, and it is also soluble in sodium bicarbonate solutions, certain organic amines and quaternary ammonium bases, but is insoluble in water and common organic solvents. Salts can be formed by controlled neutralization with base or by treatment with solutions of metallic acetates[1,2]. The property of ready formation of metallic salts and the reported adsorption of ACTH from solution[3], indicate that oxidised cellulose should serve as a useful ion exchange medium in analytical separations by functioning as a carboxylic cation exchange medium. This note will describe the application of oxidised cellulose as an ion exchange resin to alkaloidal analysis.

Experimental

Reagents. Oxidised cellulose in powder form with a carboxyl content of from 16 to 22 per cent., atropine sulphate, quinine sulphate, codeine sulphate and strychnine sulphate. 0·1 and N hydrochloric, sulphuric and acetic acids. Glass or cotton wool (washed), ethanol 95 per cent., chloroform B.P. and sodium hydroxide 3 per cent. aqueous solution.

Apparatus. Unicam SP 500 or similar spectrophotometer. Polyethylene or pyrex tubes 12 cm. long, 1 to 1·2 cm. in diameter.

Preparation of columns. Columns 5 cm. in length and 1 cm. in diameter were prepared by slurrying approximately 2 g. of oxidised cellulose with water and transferring to a polyethylene or pyrex tube plugged at one end with glass or cotton wool and fitted with a stopcock. After allowing the columns to settle, no pressure or packing being used, a small plug of cotton or glass wool was pressed on to the top of each column.

Adsorption. An aliquot of the alkaloidal solution of from 2 to 5 ml. was transferred to the top of the cellulose column and gently forced in by air pressure, this was followed by 5 ml. washes of distilled water until the pH of the effluent had returned to initial value or gave a negative sulphate reaction.

Factors Influencing the Adsorption Step

Solvent. Of the four alkaloids investigated all were quantitatively adsorbed from aqueous solutions as well as from solutions in 50 per cent. and 90 per cent. ethanol.

Rate of flow. When using 5 ml. aliquots containing 20 mg. of alkaloid and a flow rate of from 0·5 ml. per minute to 2·0 ml. per minute, there was quantitative adsorption on columns 5 cm. in length and 1·2 cm. in diameter.

Presence of other salts. 5 ml. aliquots of a solution of quinine sulphate containing varying amounts of sodium chloride were transferred to columns 5 cm. in length and 1·2 cm. in diameter and adsorbed in the usual manner. The columns were washed with a total of 50 ml. each of distilled water. The adsorbed alkaloid was then eluted with 0·1N sulphuric acid and after dilution measured spectrophotometrically at 315 mμ.

Table I shows that for a given length of column the adsorption of alkaloid is dependant both on the total salt concentration of the solution and on the alkaloid to salt ratio.

TABLE I

DEGREE OF ADSORPTION FROM SOLUTIONS CONTAINING ALKALOID AND VARYING AMOUNTS OF SODIUM CHLORIDE

Aliquots of 5 ml. containing		Per cent. alkaloid retained on column
mg. of Quinine	mg. Sodium chloride	
20	60	78
	40	96
	20	99
	10	100
10	60	90
	40	100
2·5	100	93·5
	80	97·5
	60	99·0
	40	97·0
	20	98·0
	10	97·0
	5	98·0

Elution

The two possible methods for the recovery of the adsorbed alkaloids were (*a*) dissolution of the cellulose in alkali which resulted in depolymerisation and liberation of the absorbed alkaloid which could then be extracted and (*b*) elution with acids of which hydrochloric or sulphuric acids at the strength of N or 0·1N were found to be effective, little difference in elution volumes between 0·1 and N acids was found. Both methods gave satisfactory results. The second, however, was more economical, easier to manipulate, and was the preferred method. After elution of alkaloids, columns washed free from acid could be used again. As many as 16 successive adsorptions and elutions have been performed on the same column over a period of one month without any apparent decrease in adsorption properties.

Removal of Impurities

If a spectrophotometric method was to be used for the final determination of the alkaloids in the eluate, unused cellulose was washed with a volume of 100 ml. of 0·1N sulphuric acid followed by distilled water until the washings were acid free. This treatment was necessary to remove most of the impurities which absorbed in the region 220 to 280 mμ, and which would otherwise interfere with spectrophotometric measurements in this region. Allowances must, however, sometimes be made for small residual absorption in this region by either using as a blank, the alkaloid free eluent or by the use of a predetermined figure.

General Method for Pure Alkaloidal Solutions.

Columns of oxidised cellulose 5 cm. in length and 1·2 cm. in diameter were prepared as previously described washing well with 0·1N sulphuric acid and water. A 5 ml. aliquot of the aqueous alkaloidal solution was transferred to the top of the cellulose column and gently forced in by air pressure. This was followed by 5 ml. washes with distilled water. The alkaloid was recovered by elution with 0·1N or 1N sulphuric acid, pressure being applied to maintain a flow rate of 1·5 to 2 ml. per minute. A volume of 50 ml. was collected.

Results

Atropine

Method. Two methods were used for the estimation of atropine in the eluent from the columns. (*a*) The eluate was made alkaline with strong ammonia and extracted several times with chloroform. The chloroform was washed with water and evaporated to dryness. The alkaloidal residue was then estimated titrimetrically. (*b*) Colorimetric assay was by the following method[4]. The eluate was diluted to a concentration of 0·05 mg. of atropine per ml. A 2 ml. aliquot was evaporated to dryness and nitrated by the addition of 0·3 ml. of fuming nitric acid which was removed by evaporation on a steam bath or hot plate. The nitrated residue was transferred to a 10 ml. standard flask by means of small amounts of dimethylformamide. 0·3 ml. of tetraethylammonium hydroxide 25 per cent. aqueous solution were then added and the solution made up to volume. After allowing to stand for 5 minutes the colour produced was measured at 540 mμ. The atropine content was calculated by reference to a standard graph. Results are given in Table II.

TABLE II

RECOVERY PER CENT. FROM A 20 MG. SAMPLE OF ATROPINE ELUTED WITH 0·1N AND N HYDROCHLORIC ACID

0·1N	N
97·5	99·0
99·0	96·0
97·5	100·0
97·5	97·5
101·0	100·0
101·0	100·0
98·0	99·0
98·0	99·0
100·0	99·0
100·0	104·0
101·0	101·0
100·0	101·0

Strychnine

Method. Strychnine eluate was estimated by dilution to 250 ml. with 0·1N sulphuric acid and measured spectrophotometrically at 255 mμ using a blank consisting of 10 ml. of alkaloid-free eluent diluted to 50 ml. with 0·1N sulphuric acid to compensate for background absorption. Results are given in Table III.

TABLE III

RECOVERY PER CENT. FROM A 5 MG.-SAMPLE OF STRYCHNINE ELUTED WITH 0·1N SULPHURIC ACID

99·0	101·0	101·0
101·0	100·0	97·0
100·5	101·0	98·0
100·0	98·0	98·0
98·0		

Quinine and Codeine

Method. Quinine in the eluate was estimated spectrophotometrically after dilution with 0·1N sulphuric acid at 278 mμ or 315 mμ. Measurements at 278 mμ required the use of alkaloid-free portions of the eluent suitably diluted, as a blank. At 315 mμ 0·1N sulphuric acid sufficed as a blank. Codeine was estimated in a

similar manner after dilution with 0·1N sulphuric acid by measurements at 278 mμ. Results are given in Table IV.

Discussion

Previous work on the ion exchange analysis of alkaloids using cationic resins such as Amberlites IR-120H or IRC-50 has met with certain difficulties in that whilst alkaloids were usually readily adsorbed from aqueous solution the quantitative recovery was difficult. Decalso, a synthetic zeolite, has been used with some success in this direction, the presence, however, of small amounts of salts interferes with adsorption. Oxidised cellulose resembles the strong cationic resins such as IR-120H in its uptake of alkaloid from solution but, unlike IR-120H, recovery of adsorbed alkaloid is rapid and quantitative, and is probably due to the lack of cross linking in the material, thus enabling the free passage of alkaloid molecules to and from the sites of exchange. Oxidised cellulose appears to be superior to the resin IRC-50, and Decalso in the uptake of alkaloid from salt solution, and should therefore be useful in the analysis of alkaloidal extracts which often contain appreciable amounts of other salts. Work in this direction is proceeding.

TABLE IV

Recovery per cent. from 5 mg. samples of quinine and codeine eluted with 0·1N sulphuric acid

Quinine	Codeine
101·0	97·0
101·0	97·0
101·0	97·0
100·0	97·5

Summary

1. Oxidised cellulose has been successfully employed as an ion exchange material in the determination of atropine, strychnine, quinine and codeine.

2. These alkaloids were readily adsorbed from solution and recovery was rapid and quantitative.

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THE PHARMACOLOGY OF NICOTINE MONOMETHIODIDE

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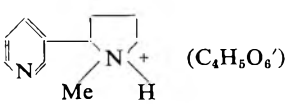
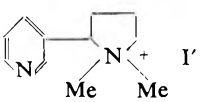
Introduction

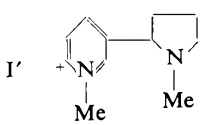
Nicotine is a ditertiary base. It forms two monomethiodides and a dimethiodide (Fig. 1). Nicotine is of great interest because, although it does not contain an "-onium" group, it both stimulates and then blocks the ganglionic and neuromuscular synapses. Nicotine dimethiodide (NDM) was shown by Crum-Brown and Fraser in 1869[1] to paralyse a frog nerve-muscle preparation in a curare-like manner and to be much less toxic than the parent alkaloid, nicotine. Nicotine dimethiodide has two quaternised nitrogen atoms and neuromuscular blocking activity might not be unexpected.



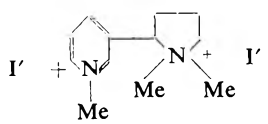
Nicotine Hydrogen Tartrate (NHT)



Nicotine Monomethiodide (NMI)



Nicotine *iso*Methiodide (NIM)



Nicotine Dimethiodide (NDM)

Fig. 1.

Nicotine *iso*methiodide (NIM) and NDM in 5 mg. doses were shown by Burn[2] not to raise the blood pressure of the spinal cat. Nicotine monomethiodide (NMI), a crystalline, but extremely hygroscopic substance, which contains one molecule of water of crystallisation, was prepared by Barlow and Dobson in 1955[3]. Barlow and Dobson[3] also made a preliminary study of the pharmacological properties of this compound. In two experiments on the blood pressure of the spinal cat, they found that NMI was roughly as active as nicotine hydrogen tartrate (NHT), although in one other experiment, it was found to have only 40 per cent. of the pressor activity of the latter. Barlow and Dobson[3] also pointed out that the pressor response to NMI differed in form from that to NHT.

Some degree of sensitisation to the monomethiodide was shown. 140 mg. of NMI in doses of 10 mg. given over a period of one hour, did not produce complete ganglion block but the pressor effect of a subsequent dose of 1 mg. of NHT was reduced. Prior injection of 0·25 mg. of hexamethonium bromide almost abolished the pressor effects of 0·6 mg.

NHT and of 0·6 mg. NMI. Taylor[4] has calculated from the dissociation constants of nicotine, that it exists at body pH, as the univalent nicotinium ion. In this form, the cationic head—N^+MeH—is presented to the receptor surface by the pyrrolidine ring. Barlow[5] points out that if the active species is the nicotinium ion then the corresponding *N*-methyl quaternary salt (NMI) would be expected to be much more potent than nicotine itself. When the pyridine nitrogen is quaternised as in NIM, the activity should be much reduced.

This account deals with some aspects of the pharmacology of NMI, NDM and NIM supplied by Drs. Barlow and Dobson.

All drug concentrations refer to final bath concentrations.

Experimental Methods and Results

Guinea-pig Terminal Ileum. A strip of guinea-pig terminal ileum, about 2 cm. long, was suspended in a 2 ml. bath containing oxygenated Tyrode's solution at 33° C. Nicotine monomethiodide stimulated the gut at doses of 5 to 500 μg./ml. without producing tachyphylaxis. At the beginning the response to NMI was small, but as the experiment proceeded and other active drugs (histamine and acetylcholine) were used, the NMI response increased to a constant level after about an hour. This response to a given dose was maintained throughout the rest of the experiment. Tested over a dose range of 25 to 500 μg./ml. NHT and NMI appeared to be equipotent. The contractions induced by 100 μg./ml. NMI were completely inhibited by 50 μg./ml. papaverine sulphate (Fig. 2), which on the same preparation inhibited the responses to 25 mg./ml. of barium chloride and 1 μg./ml. of acetylcholine bromide. It was much more difficult to obtain constant, reproducible responses on the ileum to NHT than to NMI. 0·25 μg./ml. atropine sulphate inhibited the response to 250 μg./ml. NMI (Fig. 3). 250 μg./ml. of hexamethonium bromide considerably reduced but did not completely eliminate the response to 100 μg./ml. NMI (Fig. 4). 25 μg./ml. to 1 mg./ml. NIM and 50 μg./ml. to 1 mg./ml. NDM did not stimulate the ileum.

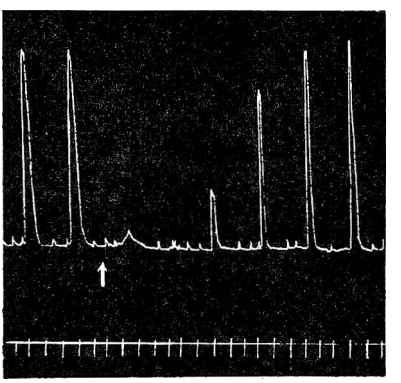


Fig. 2. Inhibitory effect of papaverine on NMI contractions of the guinea-pig terminal ileum. 100 μg./ml. NMI added every 2 minutes. At arrow, 50 μg./ml. of papaverine sulphate was added 1 minute before NMI. Time signal = 1 minute.

Virgin Rat Uterus. Œstrus was induced in rats weighing between 185 and 220 g. by means of a subcutaneous injection of 10 μg./100 g. body weight of stilbœstrol in arachis oil, 24 hours before use. One horn of the uterus was suspended in a 2 ml. bath containing oxygenated de Jalon's solution at 29° C. No direct action was seen with doses of NMI

from 25 to 500 μg./ml., NDM from 500 μg. to 1 mg./ml., NIM from 500 μg. to 1 mg./ml., or NHT from 100 μg. to 1 mg./ml.

Frog Sartorius Muscle—Ischiad Nerve Preparation. This preparation was used in a 50 ml. bath containing oxygenated Frog Ringer's solution at room temperature. The nerve was stimulated by means of a square

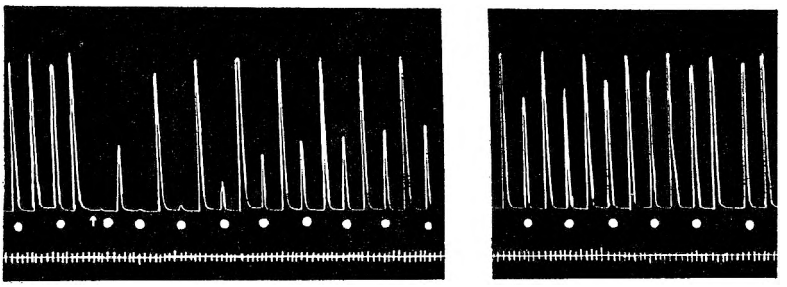


FIG. 3. Inhibitory effect of atropine on NMI contractions of the guinea-pig terminal ileum. At dots 250 μg./ml. NMI added. Unmarked responses due to 0·25 μg./ml. of acetylcholine bromide. At arrow, 0·25 μg./ml. of atropine sulphate added 1 minute before NMI. Time signal = 30 seconds.

wave stimulator delivering 7 stimuli per minute. The duration and voltage of the impulse were 1·1 millisecs. and 40 volts respectively. 5 μg./ml. NMI produced a slight potentiation of the response with no inhibition. 10 μg./ml. NMI caused slight inhibition, followed by slight potentiation of the response. 40 μg./ml. NMI caused spontaneous twitching and a fall in tone. At this dose level there was complete inhibition of the contractions (Fig. 5), which was reversible on washing.

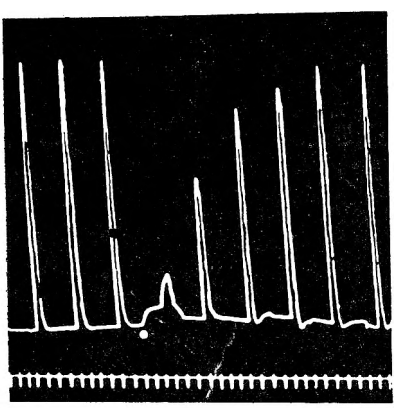


FIG. 4. Inhibitory effect of hexamethonium on NMI contractions of the guinea-pig terminal ileum. 100 μg./ml. NMI added at 2-minute intervals. At dot, 250 μg./ml. hexamethonium bromide added 1 minute before NMI. Time signal = 1 minute.

Rat Phrenic Nerve—Diaphragm Preparation. This preparation was suspended in a 100 ml. bath containing oxygenated Tyrode's solution with double the normal amount of glucose. The muscle was stimulated indirectly by the nerve and also directly by means of a square wave stimulator delivering 7 stimuli per minute. The duration and voltage of the impulse were 1·1 millisecs. and 40 volts respectively. 5 to 10 μg./ml. of NMI produced neither potentiation nor inhibition of the response to indirect stimulation of the muscle. Doses of from 20 to 60 μg./ml. NMI gradually inhibited the response. There was a roughly graded relation between the dose and the degree of inhibition. When indirect

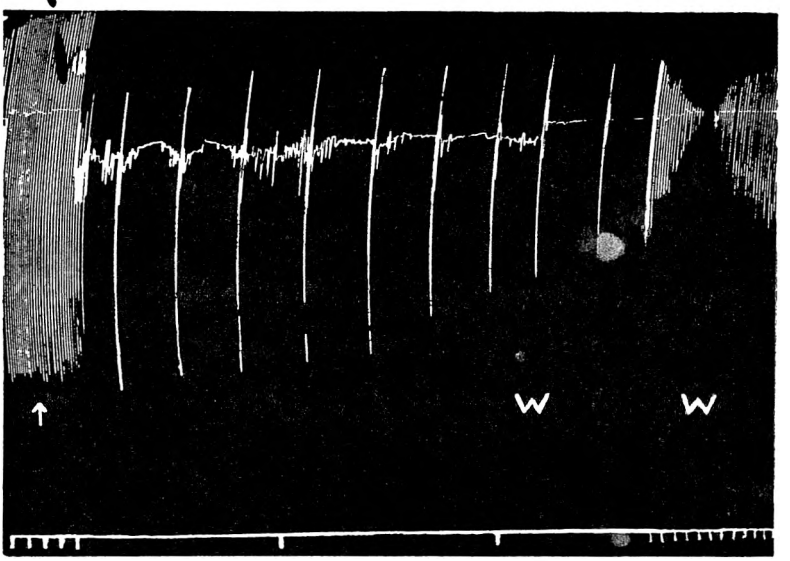


FIG. 5. Effect of NMI on neuromuscular conduction in the frog sartorius —ischiad preparation. At arrow, 40 μg./ml. NMI added. At W, bath emptied and refilled with fresh frog ringer's solution. Time signal = 1 minute.

stimulation failed to produce a response, direct stimulation of the muscle caused a small contraction (Fig. 6). NMI appeared to be about twice as potent as NHT in inhibiting the electrically induced contractions of the rat diaphragm. Doses of 50 to 250 μg./ml. NDM and NIM had no observable effect on this preparation.

Frog Rectus Abdominis Muscle. The frog rectus abdominis muscle strip was suspended in a 10 ml. bath containing oxygenated Frog Ringer's solution at room temperature. NMI had a stimulant action on the rectus and gave graded and reproducible responses to doses of from 0·05μg. to 2·5 μg./ml. There was no twitching and no sign of tachyphylaxis. On this preparation, NHT was always found to be slightly less potent than NMI. NDM at doses of from 50 μg. to 500 μg./ml. had a stimulant action and produced graded and reproducible

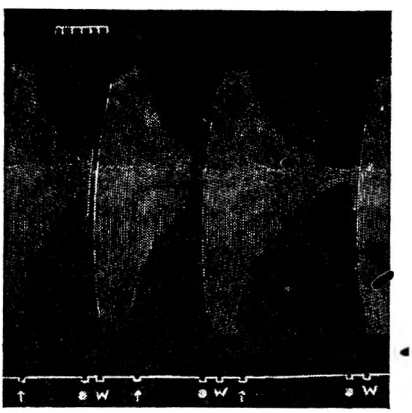


FIG. 6. Effect of NMI on neuromuscular conduction in the rat phrenic nerve-diaphragm preparation. At arrow, 50 μg./ml. NMI added to bath. At W, bath emptied and refilled with fresh, double glucose, Tyrode's solution. At dot, direct, electrical stimulation of diaphragm. Time signal = 1 minute.

responses between those doses. 0·5 mg. to 1·0 mg./ml. NIM produced no response on this preparation.

Perfused Frog's Heart. Perfusion was made through the *sinus venosus* with oxygenated Frog Ringer's solution at room temperature. Drugs were administered in solution, by injection into the side arm of the perfusion cannula. Doses of from 0·5 μg. to 1 mg. NMI in 1 ml. had no noticeable effects, but 2 mg. caused an increase in amplitude and 5 mg. a marked increase in amplitude with a slight slowing of the heart.

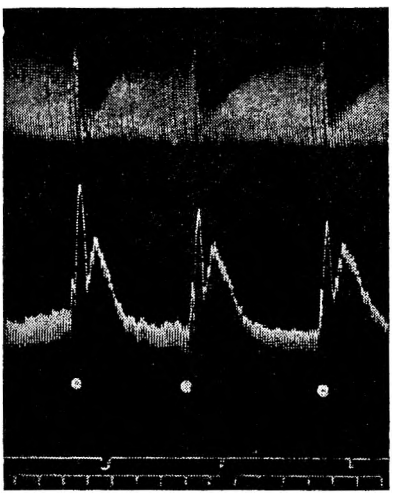


FIG. 7. Chloralosed cat: upper record, respiration, lower record, blood pressure. At dot, 0·25 mg. NMI + 3 ml. saline added. Time signal = 30 seconds.

Blood Pressure and Respiration of the Chloralosed Cat. Anæsthesia was induced in cats weighing between 2·75 and 3·5 kg. with ether or an ether-chloroform mixture and maintained with chloralose. Drugs were administered into a cannula inserted into the external jugular vein and arterial blood pressure was recorded from the carotid artery in the usual way. To record respiration, a thread was sewn into the abdominal wall and led over pulleys to a suitable recording lever. Cats varied in their response to NMI. A dose of NMI which in one cat would produce a large rise in blood pressure, with marked muscle twitching, respiratory depression and a short period of apnœa would, in another, cause only a slight pressor effect with a momentary depression of respiration. NMI appeared to have a cumulative effect on respiration, and after a series of doses it was usually necessary to give artificial respiration. In the majority of cats, spontaneous respiration appeared to be irreversibly inhibited and artificial respiration had to be given for the remainder of the experiment. The characteristic effects of 0·5 mg. of NMI in a 3 kg. cat were: very slight stimulation followed by marked depression of respiration with apnœa. If spontaneous respiration recovered, the depth was usually markedly increased. We did not observe the marked respiratory stimulation which followed NHT. The effects on blood pressure of doses of NMI of from 0·1 to 1·0 mg. appeared to be graded and often showed a secondary, more prolonged rise (Fig. 7). 0·25 mg. ergotoxine ethanosulphonate reduced the height of the response to NMI and where there was a secondary rise, this was inhibited. 0·15 mg. Hydergine reversed the pressor effects of 0·4 mg. NMI. 1 mg. of hexamethonium bromide almost abolished the pressor response to a similar dose of NMI. 1 mg. tetra-ethylammonium bromide reduced, but did not abolish the pressor response to 0·2 mg. NMI (Fig. 8) and the pressor effects of 1·5 mg. NMI were partly blocked

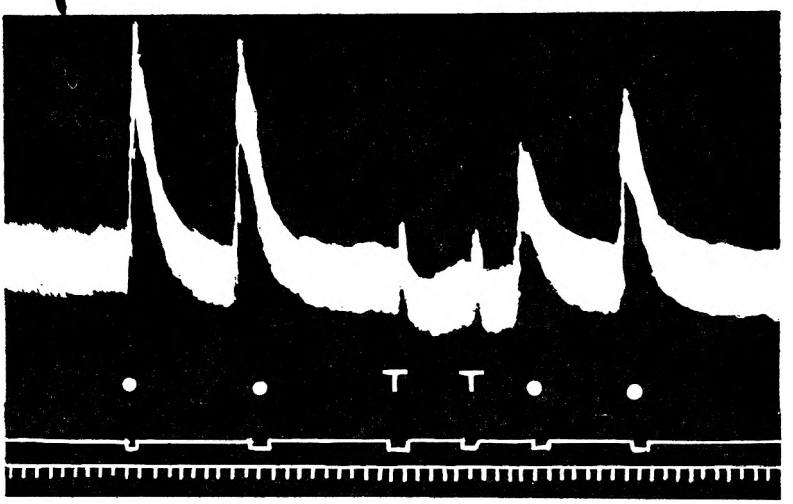


FIG. 8. Inhibitory effect of TEA on NMI pressor response. Chloralosed cat: blood pressures. At dots, 0·2 mg. NMI + 3 ml. saline added. At T, 1·0 mg. tetra-ethyl ammonium bromide + 3 ml. saline added. Time signal = 30 seconds.

by 1 mg. atropine sulphate (Fig. 9). In one cat only, a 1 mg. dose of NMI produced a diphasic depressor-pressor response. NMI appeared to have from 40 to 80 per cent. of the pressor activity of NHT in this series of experiments. It was more difficult to produce tachyphylaxis to

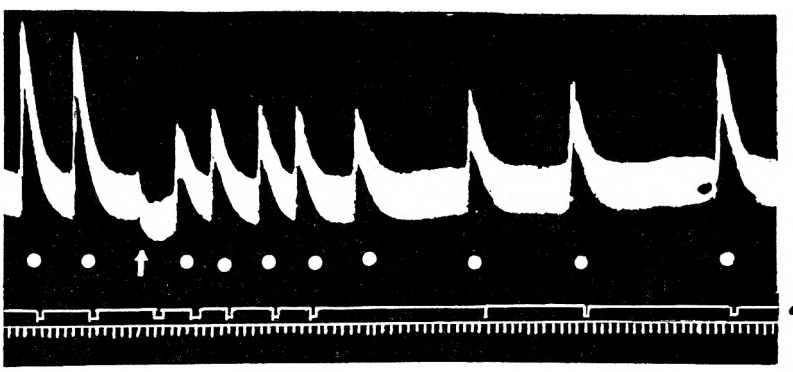


FIG. 9. Effect of Atropine on NMI pressor response. Chloralosed cat: blood pressure. At dots, 1·5 mg. NMI + 3 ml. saline added, at arrow, 1·0 mg. atropine sulphate + 3 ml. saline. Time signal = 30 seconds.

NMI on this preparation than to NHT. In one preparation, for example, tachyphylaxis was produced by giving eight 0·5 mg. doses of NHT over a period of 5 minutes. On the same preparation, however, fourteen 0·5 mg. doses of NMI had to be given in 5 minutes to produce a similar effect. 25 mg. NDM produced a slight depressor response and 25 mg. NIM produced a diphasic depressor-slight pressor response.

Blood Pressure of the Spinal Cat. Once again the magnitude of the pressor response varied from cat to cat. The pressor response to 1 mg. NMI could be completely blocked by 1 mg. of hexamethonium bromide (Fig. 10). NMI appeared to have about 40 per cent. of the potency of

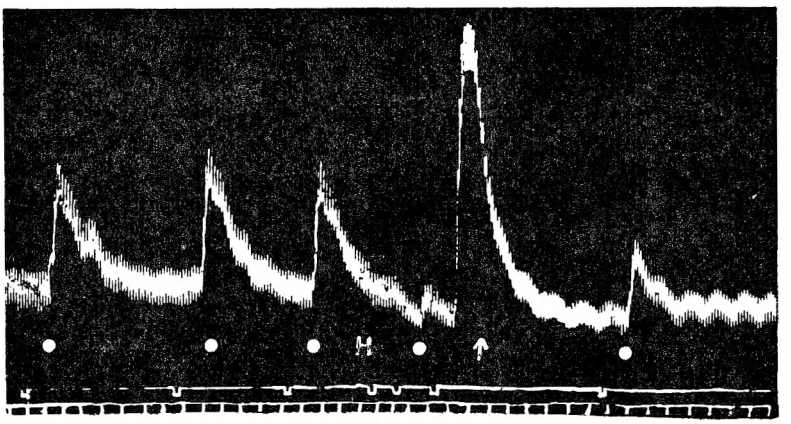


FIG. 10. Inhibitory effect of hexamethonium on NMI pressor response. Spinal cat: blood pressure. At dot, 1 mg. NMI + 3 ml. saline added; at H, 1 mg. hexamethonium bromide in 3 ml. saline added, at arrow; 5 μg. adrenaline + 3 ml. saline added. Time signal = 1 minute.

NHT on this preparation (Fig. 11). It was difficult to produce ganglion block. In one instance, 70 mg. NMI in 5·0 mg. doses was effective when given in the space of 3·5 minutes (Fig. 12). Recovery was rapid. 10 mg. of NDM and 5 mg. of NIM had no effect on the blood pressure of the spinal cat.

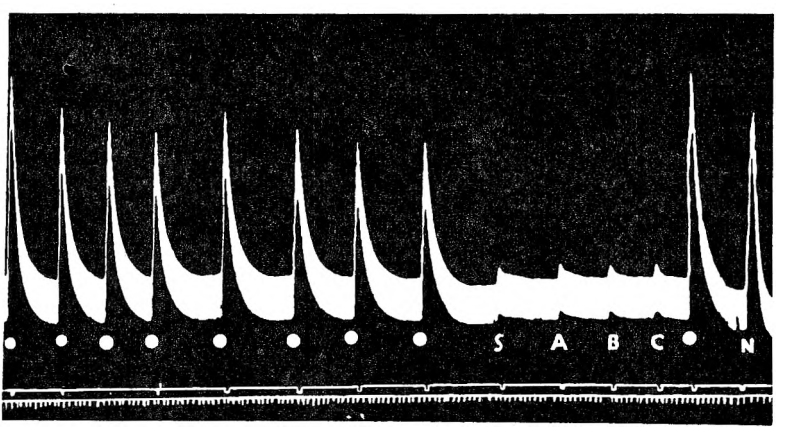


FIG. 11. Spinal cat: blood pressure. At dots, 5 mg. NMI + 3 ml. saline added; at S, 3 ml. saline added; at A, 1 mg. NDM + 3 ml. saline added; at B, 3 mg. NDM + 3 ml. saline added; at C, 5 mg. NDM + 3 ml. saline added; at N, 2 mg. NHT + 3 ml. saline added. Time signal = 30 seconds.

Nictitating Membrane of the Chloralosed Cat. 1 mg. of NMI produced a contraction of the nictitating membrane. In one preparation, when 30 mg. NMI were given over a period of 4 minutes in 5 mg. doses, stimulation and then block of the superior cervical ganglion was produced.

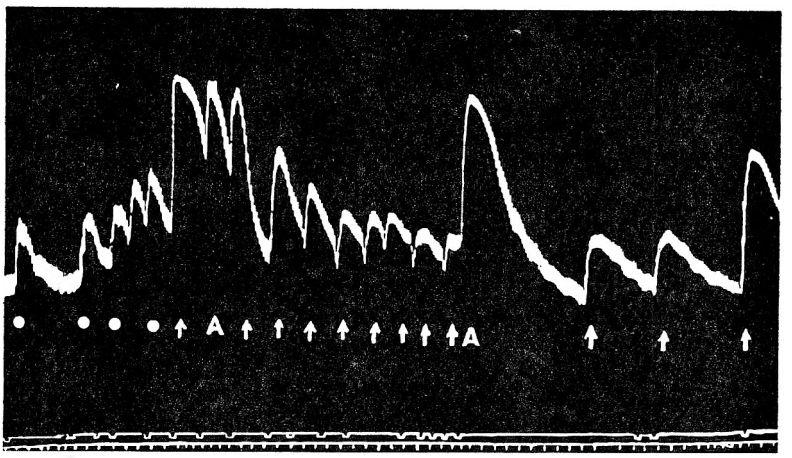


FIG. 12. Ganglion block produced by NMI. Spinal cat: blood pressure. At dots, 1 mg. NMI + 3 ml. saline added; at arrows, 5 mg. NMI + 3 ml. saline added; at A, 10 μg. adrenaline + 3 ml. saline added. Time signal = 30 seconds.

CONCLUSIONS

On skeletal muscle NMI appears to be somewhat more potent than NHT. It has a slightly greater stimulant action on the frog rectus and depresses the rat diaphragm more effectively than NHT at similar doses. NMI is a more potent respiratory depressant than NHT and seems to have a cumulative effect on respiration. NMI is less active in both stimulating and depressing the autonomic ganglia which influence the blood pressure. Although it may be more firmly held on to the receptor surfaces[5], the nicotinic activity of NMI does not appear to be increased, at any rate at the ganglionic synapse. In stimulating frog skeletal muscle, there is little difference in the potency of the two compounds; on the other hand NMI is much more effective in depressing the rat diaphragm. These differences may be a reflection of varying degrees of accessibility of the receptors at these different sites to the quaternary compound, or there may be differences in the susceptibility to enzymic attack. It is worth speculating whether the normal metabolism of nicotine includes a quaternary compound, at an early stage. This would be an analogue of the monomethiodide. Possibly one of the hydrogen atoms on the pyrrolidine nitrogen is replaced by another group. In NMI a methyl group has replaced this hydrogen atom. This may make it more susceptible to further attack. The next step might be the formation of a compound analogous to the *iso*methiodide which we have found to be completely devoid of nicotinic properties. The dimethiodide still possesses slight nicotinic activity on the blood pressure of the chloralosed cat and on the

frog rectus abdominis, but it is very much less active than either the hydrogen tartrate or the monomethiodide.

The inhibitor action of atropine towards the NMI-induced contractions of the guinea-pig ileum (Fig. 3), and the NMI-induced pressor response of the chloralosed cat (Fig. 7) is interesting. This effect was not seen with NHT.

Summary

1. Nicotine monomethiodide had a direct stimulant action on guinea-pig ileum and frog rectus abdominis muscle which with ileum was antagonised by atropine and hexamethonium.

2. The pressor response to nicotine monomethiodide of the chloralosed and spinal cat was qualitatively similar to that of nicotine hydrogen tartrate and could be modified or abolished by atropine, tetra-ethyl ammonium or hexamethonium.

3. The effect of nicotine monomethiodide on respiration appeared to be cumulative and was characterised by slight initial stimulation followed by depression and then apnœa. This effect differed from that of nicotine hydrogen tartrate.

4. Nicotine monomethiodide was more potent in depressing the rat phrenic nerve diaphragm preparation than nicotine hydrogen tartrate and in very large doses increased the amplitude of the perfused frog heart.

5. The possible significance of the varying nicotinic activity in the series nicotine hydrogen tartrate, nicotine monomethiodide, nicotine dimethiodide and nicotine *iso*methiodide is discussed.

We wish to thank Messrs. Sandoz, Ltd., for kindly supplying us with a sample of Hydergine; Mr. R. E. Lister for help with the rat diaphragm; Mr. R. Callander for Fig. 1; and we are grateful for the technical assistance of Miss Iris Topping and Miss Elizabeth Duff.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Canescine, Isolation and Structure of. M. W. Klohs, F. Keller, R. E. Williams and G. W. Kusserow. (*J. Amer. chem. Soc.*, 1955, **77**, 4084.) Canescine ($C_{32}H_{38}O_8N_2$), a new alkaloid, was isolated from the crude reserpine fraction obtained from the roots of *Rauwolfia canescens* by the application of chromatography using acid washed alumina as the adsorbent. On rapid crystallisation from methanol it was obtained as fine needles whereas slow crystallisation yielded prisms, both forms melting at 232–234° C., $[\alpha]_D^{24}$ $-138 \pm 2°$ (*c* 1·0 in chloroform). Ultra-violet data is recorded. On basic hydrolysis with dilute methanolic sodium hydroxide, canescine yielded canescic acid and one molecule of 3 : 4 : 5-trimethoxybenzoic acid. Selenium dehydrogenation of methyl canescate yielded yobyrine. A tentative structural formula for canescine is proposed. The new alkaloid has the same order of sedative activity as reserpine and hypotensive activity comparable to reserpine and rescinnamine.

A. H. B.

***Datura innoxa*, New Alkaloid From.** E. Steinegger and F. Gessler. (*Pharm. Acta Helvet.*, 1955, **30**, 279.) Paper chromatography of the alkaloids from the root of *Datura innoxa* Miller indicated the presence of an alkaloid with an R_F of 0·9. This was obtained only from plants in the later stages of development. The base had m.pt. of 44° to 46° C., and the picrate 77° to 78° C.

G. M.

Recanescine, a New Sedative Principle of *Rauwolfia canescens* Linn. N. Neuss, H. E. Boaz and J. W. Forbes. (*J. Amer. chem. Soc.*, 1955, **77**, 4087.) Recanescine ($C_{32}H_{38}O_8N_2$) was isolated by chromatography of the mother liquor from crystallisation of reserpine on acid-washed alumina using benzene as eluent. Hydrolysis of the alkaloid yielded 3 : 4 : 5-trimethoxybenzoic acid and methyl recanescate, characterised as a tosyl ester. Reductive cleavage of recanescine gave recanescic alcohol and 3 : 4 : 5-trimethoxybenzyl alcohol. Infra-red and ultra-violet data are given; from this and other evidence the structure of recanescine as 11-desmethoxyreserpine is suggested. Recanescine has the same pharmacological properties as reserpine in monkeys and rabbits (sedation, myosis and ptosis) and anæsthetised cats and dogs (blood pressure lowering, apparent enhancement of the pressure response to adrenaline and inhibition of blood pressure response to bilateral carotic occlusion).

A. H.B.

ANALYTICAL

Aneurine and Riboflavine, Estimation of. K. V. Giri and S. Balakrishnan. (*Analyt. Chem.*, 1955, **27**, 1178.) Circular paper chromatography is used for the separation and estimation of aneurine and riboflavine in multivitamin preparations. Solutions containing the vitamins were spotted on the circumference of a 2·2-cm. circle at the centre of an 18-cm. Whatman No. 1 paper. While still damp, the paper was exposed for about 20 minutes to the vapours of cyanogen

bromide and ammonia contained in two petri dishes; when dry it was removed, and a cylindrical paper wick was placed at its centre. It was then developed using 1-butanol-acetic-acid-water until the solvent boundary had run almost to the edges of the circle, when the chromatogram was removed, air-dried, and observed under ultra-violet light. The fluorescent bands of thiochrome and riboflavine, which showed distinctly on the chromatogram, were cut and eluted with 6 ml. of water and shaken in extraction cylinders. The fluorescence of the solutions was measured in a Klett fluorimeter against water blanks using the necessary filters for aneurine and riboflavine. Standard curves for amounts of vitamins ranging from 0·56 to 2·24 μg. were linear and replicate determinations gave a reproducibility of about 10 per cent. The thiochrome band was stable on the paper but the riboflavine began to deteriorate after 14 days. Good recoveries were obtained in the analysis of multivitamin preparations. R. E. S.

Atropa Alkaloids, Colorimetric Determination of. F. M. Freeman. (*Analyst*, 1955, **80**, 520.) An experimental study has been made of the Vitali Morin colour reaction between acetone and aromatic nitro-compounds in the presence of sodium hydroxide. With acetone or pyridine as the solvent, and ethanolic potassium hydroxide as the base, the colour stability was poor, being critically dependent on the water content of the reaction mixture. Sodium methoxide in benzene-methanol was found to be superior to ethanolic potassium hydroxide but the most promising results were obtained by the use of tetraethylammonium hydroxide with dimethylformamide as the solvent. In the final procedure 0·05 to 0·15 mg. of the alkaloid was nitrated with 0·2 to 0·3 ml. of fuming nitric acid and, after evaporation, 0·3 ml. of 25 per cent. aqueous tetraethylammonium hydroxide and dimethylformamide were added. After setting aside for 5 minutes the extinction at 540 mμ was measured. Graphs of colour stability and times are given for two hyoscyamine solutions; reaction mixtures containing up to 10 per cent. of water showed little change in the rate of fading compared with mixtures containing below 0·1 per cent. The reaction was also applied successfully to the determination of phenylacetic acid, benzylpenicillin and dibenzylethylenediamine penicillin. R. E. S.

Copper in Plant Materials, Determination of. S. Andrus. (*Analyst*, 1955, **80**, 514.) A method is described for the rapid absorptiometric determination of copper in plant materials. Organic matter is destroyed by digestion for 30 minutes with sulphuric, nitric and perchloric acids until colourless followed by heating to fuming with hydrogen peroxide (100 volume). The copper, after dilution of the acid solution, is extracted with a solution of zinc dibenzyldithiocarbamate in carbon tetrachloride, and the extinction measured at a wavelength of 440 mμ against a blank determination; the amount of copper present is obtained from a standard curve prepared using a solution of copper sulphate. The method was free from interference by other metals commonly present in plants and amounts of copper varying from 10 to 40 μg. were satisfactorily recovered in the presence of 1000 μg. quantities of iron, cobalt, manganese and molybdenum. A study of the digestion procedure showed that there was no loss of copper and in experiments in which 5·0 p.p.m. copper were added, amounts varying from 4·9 to 5·3 were recovered. R. E. S.

Hydrocortisone, Determination of. C. R. Szalkowski, M. G. O'Brien and W. J. Mader. (*Analyt. Chem.*, 1955, **27**, 944.) A method is given which is based on the yellow colour produced by hydrocortisone in a mixture of sulphuric and glacial acetic acids; the colour, with a maximum absorption at 470 mμ can,

be used for the identification and colorimetric determination of hydrocortisone when mixed with cortisone, and in preparations. The colour produced by hydrocortisone in sulphuric acid showed a maximum at 390 mμ and a broad band between 445 and 475 mμ; the addition of glacial acetic acid produced a lower 390 mμ maximum with a peak at 470 mμ. All absorptions were measured after 1 hour. The test distinguished between hydrocortisone and cortisone since with cortisone the absorption at 470 mμ was negligible. The absorption values for 30 additional steroids in sulphuric acid and in sulphuric/acetic acids are recorded. Hydrocortisone, corticosterone, 21-hydroxypregnanetrione-3:11:20-œstradiol, œstrone, and stilbœstrol produce a fluorescent yellow colour with the mixed acid reagent suggesting that the fluorescence reaction can be used for identification purposes. A procedure for the determination of hydrocortisone in ointments is given; for an ointment made to contain 10 mg. of hydrocortisone per g. the standard deviation for 12 assays was 0·2 mg. per g. with an average of 10·2 mg. per g. R. E. S.

Opium Alkaloids, Paper Chromatography of. J. Reichelt. (*Pharmazie*, 1955, **10**, 234.) The method employed has been described previously by the author (*Pharmazie*, 1954, **11**, 968). The paper is impregnated with a mixture of formamide and ethanol (1 + 1), and the reproducibility of the results depends on the ratio of these compounds, the maintenance of a uniform process of impregnation, the temperature during the development and the degree of saturation of the chamber by the solvent vapour. Solvents used for development are benzene-chloroform (2 + 3 parts by volume) and benzene. Illustrations are given of the separation, with the former mixture, of mixtures of thebaine, heroin and papaverine, of morphine, codeine, ethylmorphine and dihydroxycodeinone, and of ethylmorphine and codeine in a ratio of 1:30. With benzene, a separation of codeine, dionine, dicodide and eucodal may be attained. Dragendorff's reagent is used for making the spots visible. Separation of dicodide and eucodal is not very good, since the spots are too close together. The method is not suitable for the identification of morphine, dilaudid and cotarnine, which remain at the starting point. It may be employed for the identification of alkaloids in pharmaceutical preparations (tablets, etc.), after a preliminary extraction of the alkaloids. G. M.

Surface-Active Agents Containing Polyoxyethylene or Polyoxypropylene Group, Detection of. M. J. Rosen. (*Analyt. Chem.*, 1955, **27**, 787.) It was found that all types of compounds containing the polyoxyethylene group could be detected by pyrolysis in 85 per cent. phosphoric acid, the volatile products being led into an aqueous solution of sodium nitroprusside containing a water-soluble secondary amine, such as diethanolamine; under these conditions the polyoxyethylene group decomposes to yield acetaldehyde which produces a blue colour with the sodium nitroprusside and the secondary amine. The test could also be used to detect the polyoxypropylene group; here the polyoxypropylene group decomposes under the conditions of the test to yield propionaldehyde (which can be isolated as the 2:4-dinitrophenylhydrazone from the water-soluble fraction of the pyrolysis products) and its polymers, which produce orange colours with sodium nitroprusside and diethanolamine. Positive results were obtained in the presence of the ester, alkylaryl, sulphide, sulphonate, sulphate, amino, amido, and phosphate groups. Glycerides interfered under the conditions of the test, decomposing to acrolein, which also gives a blue colour with sodium, nitroprusside and diethanolamine. Colours are described for a large number of surface active agents. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Cholinesterase Standards. J. H. Fleisher, S. Spear and E. J. Pope. (*Analyt. Chem.*, 1955, **27**, 1080.) The preparation of cholinesterase standards, which are stable for as long as 6 months, is described. A 1 per cent. solution of the enzyme is made in a stabilising medium containing potassium chloride, bovine hæmoglobin, bovine albumin, and phosphate buffer adjusted to pH 7·4; this is spotted on to filter paper discs which are cut out and dried. Four methods are given for the determination of the enzyme using the discs. In the colorimetric method each disc is eluted in potassium chloride solution, aliquots of eluate being incubated with acetylcholine-phosphate solution for 10 minutes at 25° C., and the residual acetylcholine being determined colorimetrically by the Hestrin (*J. biol. Chem.*, 1949, **180**, 249), procedure. For the electrometric method each disc is eluted in buffer solution and at known intervals, acetylcholine is added and the initial pH is taken followed by incubation at 25° C., and reading of the pH value after exactly 60 minutes. In the titrimetric method discs are eluted with potassium chloride-gelatin solution at pH 7·4, acetylcholine is added followed by small volumes of standard sodium hydroxide to maintain the pH at 7·4, the volumes of sodium hydroxide and the time added being recorded. In the manometric method, a disc prepared with the 0·2 per cent. enzyme is cut into several thin ribbons which are placed in the main section of a Warburg vessel and covered with 2 ml. of 0·025M bicarbonate buffer, pH 7·4, containing 0·03M magnesium chloride. The side arms receive 0·2 ml. of 0·11M acetylcholine. The vessels are attached to their manometers, which are then transferred to the water bath at 25° C. and gassed with 5 per cent. carbon dioxide-95 per cent. nitrogen for 15 minutes while shaking. The remainder of the procedure is carried out in the usual manner. The carbon dioxide output in microlitres was plotted against time, and found to be linear throughout the 60-minutes interval studied. The cholinesterase activity found by these four procedures varied by 2 to 3 per cent. and the discs were considered to be satisfactory and reproducible standards. R. E. S.

Noradrenaline and Adrenaline, Free and Conjugated, in Urine, Diurnal Variations in. U. S. von Euler, S. Hellner-Björkman and I. Orwén. (*Acta physiol. scand.*, 1955, **33**, Suppl. 118, 10.) Determining the 24 hour urinary excretion of noradrenaline and adrenaline in healthy adults, a mean value of 27 μg. for noradrenaline and 4·3 μg. for adrenaline was found. The output of both amines was considerably reduced during the night. The total catechol amines (i.e. free and conjugated) showed approximately the same distribution as the free amines but were about twice the amount. There was no difference between the sexes. M. M.

BIOCHEMICAL ANALYSIS

Adrenaline and Noradrenaline, Fluorimetric Micromethod for Differential Estimation of. U. S. von Euler and I. Floding. (*Acta physiol. scand.*, 1955, **33**, Suppl. 118, 45.) Previous fluorimetric methods for the estimation of adrenaline and noradrenaline are insufficiently specific for these two amines when biological material is tested. Modifying Ehrlén's method, a differential oxidation of adrenaline and noradrenaline with potassium ferricyanide at pH 3·5 and

6·0 is achieved. By addition of zinc sulphate complete oxidation of adrenaline is achieved in a few minutes at pH 3·5 while only some 2–4 per cent. of the noradrenaline is oxidized in the same time. Fading of the fluorescence in alkali is prevented with ascorbic acid. M. M.

Bromide in Body Fluids, Determination of. G. Hunter. (*Biochem. J.*, 1955, **60**, 261.) A micromethod for the estimation of bromide in presence of chloride and in the absence of organic matter is based on the quantitative conversion of bromide into bromate by hypochlorite; bromine is then liberated under suitable conditions followed by quantitative conversion to tetrabromorosaniline which is measured spectrophotometrically. The organic matter in 1 ml. blood was destroyed with a negligible carbon residue by ignition at 600° C. for 30 min.; under these conditions loss of bromide by volatilisation varied between 2 and 5 per cent. Amounts of bromine as low as 1 μg. can be determined with a standard deviation of less than 5 per cent. and an extreme range of variation of less than 10 per cent. of the mean. Values found for the distribution of bromide in a blood were: whole blood 1·26 mg. per 100 ml., corpuscles 0·98 mg. per 100 ml., plasma 1·43 mg. per 100 ml. R. E. S.

Œstriol, Œstrone and Œstradiol in Urine, Determination of. J. B. Brown. (*Biochem. J.*, 1955, **60**, 185.) A new chemical method is described for the separate estimation of œstriol, œstrone and œstradiol-17β in the urine of men and non-pregnant women. The method is based on that of Clayton (1949, Thesis, University of Edinburgh) and involves acid hydrolysis, ether extraction, a new phase-change purification procedure for the phenolic fraction depending on methylation of the phenol group, separation of the œstrogen methyl ethers by chromatography on alumina columns, colorimetric measurement using an improved Kober colour method, and spectrophotometric correction for interfering chromogenic material. Experimental studies are given for the hydrolysis of conjugated œstrogens, the ether extraction, the separation process, the methylation, the chromatography, and the colour development and estimation, together with details of the final method. Recovery experiments, in which known amounts of œstriol, œstrone and œstradiol-17β were added to portions of acid-hydrolysed 24 hr. male urines, yielded results between 80 and 90 per cent. even at levels corresponding to 4 μg. per day. Typical figures are shown for female and male urine and the specificity, accuracy, and sensitivity of the method are discussed. R. E. S.

Serum and other Proteins, Chromatography of. H. G. Boman. (*Nature, Lond.*, 1955, **175**, 898.) A system is described for the anion exchange chromatography of a number of differing proteins. "Dowex 2" resin, 200–400 mesh, in the chloride form was used and results are given for the chromatography of serum from a patient with prostatic cancer. The amount applied was 0·5 ml. of serum dialysed against 0·02 M tris (hydroxymethyl) aminomethane buffer at pH 7·2. Elution was carried out with 0·02 M buffer and then with stepwise increases to molarities of 0·1, 0·2, 0·4 and 1·0 of buffer, keeping the pH constant at 7·2; the protein in each fraction was followed by measurement of the extinction at 280 mμ. The different fractions from the experiment were tested also for acid phosphatase; most of the phosphatase activity (82 per cent. of the activity applied) was found in the third zone (0·2 M). The second protein fraction (eluted with 0·1 M buffer) consisted of almost pure albumin while the fourth was a well-defined γ-globulin fraction; the third fraction was probably α_1- and α_2-globulin. A commercial sample of human serum albumin was studied on a

similar column the main peak containing 52 per cent. of the amount applied was not eluted until the buffer concentration is increased to 0·4 M. Using the same chromatographic technique it was possible to purify a vegetable acid phosphatase from a crude extract. R. E. S.

CHEMOTHERAPY

Diaminodiphenoxyalkanes, Symmetrical, Schistosomicidal Activity of. C. G. Raison and O. D. Standen. (*Brit. J. Pharmacol.*, 1955, **10**, 191.) The schistosomicidal activity of a series of *pp'*-diaminodiphenoxyalkanes was assessed by oral administration to mice, guinea-pigs and rabbits infected with *S. mansoni* and *S. japonicum*. High activity was found in some 300 members of the series. The primary amines and mono-methylamines showed a peak of activity at an alkane chain length of 7 to 8. The dimethylamines were unique in showing an alternation of activity with changes in alkane chain length *n*, odd-numbered members being more active than even-numbered adjacent members; peak activity again occurred with $n = 7$. All the remaining mono- and di- alkylamines (C_2 to C_5) showed peaks at $n = 4$ and $n = 7$ to 8 and in general showed decreasing activity with increase in size of the alkyl groups on the nitrogens. The most active of the diaminodiphenoxyalkanes were several times more active than lucanthone or tartar emetic against *S. mansoni* or *S. japonicum* infections in mice. Altering the position of the amino groups from the *para* position or quaternisation of the nitrogens gave compounds with no schistosomicidal activity. G. P.

PHARMACY

NOTES AND FORMULÆ

Chlorophyll, Bacteriostatic Action of. R. Ammon and L. Wolff. (*Arzneimitt.-Forsch.*, 1955, **6**, 312.) Preliminary trials with 5 samples of chlorophyll showed that only two of these had any appreciable bacteriostatic action. These two were both water soluble preparations without added copper, one being a sodium chlorophyllin and the other a sodium magnesium chlorophyllin. Positive results were obtained with gram-positive bacteria, of which 10 different organisms were used in the tests. The concentration required was high, e.g., against *Staphylococcus London* Oxford, the chlorophyll was 833,000 times weaker than penicillin, 10,000 times weaker than streptomycin, and 333,000 times weaker than chloramphenicol. The action is bacteriostatic only and not bactericidal. G. M.

Essential Oils, Stabilisation with Antoxidants. L. E. Fryklöf. (*Farm. Revy*, 1955, **54**, 341.) The effect of the addition of antoxidants to turpentine oil has been reported previously (*Farm. Revy*, 1954, **53**, 367). These experiments have now been extended to other essential oils. In all, about 40 samples of oils of anise, bergamot, lemon, fennel, lavender, peppermint and rosemary were tested. The results showed that, under normal storage conditions, the induction period can be considerably prolonged by the addition of 0·01 per cent. of nordihydroguaieretic acid, butoxyanisole, or propyl gallate. It is however essential that the stabiliser should be added before the oil has undergone an appreciable amount of deterioration, i.e. during the manufacture. Traces of iron present in the oil may give rise to a yellow or red colouration, and the efficiency is reduced. The addition of 0·05 per cent. of citric acid or of ethylenediaminetetra-acetic acid increases the effect of the antoxidant when trace metals

are present. Storage at a low temperature (not exceeding 5° C.) is also desirable. Ethanol is ineffective in preventing oxidation, and its effect appears to depend on the preferential oxidation of the ethanol to acetaldehyde, the smell of which can be detected in oils which have been so treated. G. M.

Pyrogens in Injection Solutions. J. Kessler. (*Pharm. Acta Helvet.*, 1955, **30**, 211.) The experiments of the author relate to isotonic solutions of glucose, sodium chloride, etc., which were exposed to the air for a considerable period before the bacterial count was determined and the solution was sterilised. In testing, it is essential that the temperature and reactions of the animals should be observed for several days before injection of the solution. Some animals showed a temperature rise as great as 1° C. after a non-pyrogenic injection; and with infected, and sterilised, solutions, no correlation could be observed between degree of infection and pyrogenic reaction. Solutions which had a count of 500,000 bacteria per cent. before sterilisation sometimes gave a negative pyrogen result. On the other hand, injections which produced a considerable temperature rise in healthy guinea-pigs failed to show any unfavourable result when administered to patients. G. M.

PHARMACOGNOSY

Ergot, Determination of Alkaloids in Individual Sclerotia of. M. Hecht and W. Rumpel. (*Sci. Pharm.*, 1955, **23**, 73.) A single sclerotia is powdered with the aid of a coarse steel file, and 30 to 200 mg. of the powder is treated in a centrifuge tube with 10 ml. of ether and 1 drop of ammonia (10 per cent.) for each 25 mg. of powder. After shaking for 2 hours, and settling, 2 ml. of the solution is pipetted into another centrifuge tube and evaporated at a low temperature under vacuum. The residue is taken up in 1·0 ml. of a 4 per cent. solution of tartaric acid in 50 per cent. methanol, treated with 1 ml. of 10 per cent. zinc acetate solution, and warmed to about 35° C. After the resulting precipitate has settled, 1 ml. of the solution is treated with 2 ml. of reagent (0·2 g. of *p*-dimethylaminobenzaldehyde in a cooled mixture of 35 ml. of water and 65 ml. of concentrated sulphuric acid with 0·03 ml. of ferric chloride solution). After 20 minutes the blue colour is determined photometrically to give the total alkaloids. Another portion of 7 ml. of the ether extract is evaporated almost to dryness, then taken up with 2 ml. of 2 per cent. ammonium sulphate solution, and the remainder of the ether is removed. The solution is shaken out with 2 ml. of benzene, centrifuged, and the benzene is removed. This is repeated three times with 1 ml. of benzene each time. Turbid solutions are treated if necessary with 1 ml. of light petroleum. The water-soluble alkaloids are then determined as before, by treating 1 ml. of the aqueous solution with 2 ml. of the reagent. The results obtained show considerable variations in the alkaloid content of different sclerotia from the same strain of ergot. They are summarised in the table below. The alkaloidal contents are given as mg. per cent., calculated as ergotamine base (for total alkaloids) or ergometrine base (for water-soluble alkaloids).

Type of ergot	Total alkaloids			Water soluble alkaloids		
	Maximum	Minimum	Mean	Maximum	Minimum	Mean
Ergotoxine strain (I) ..	1200	258	811	123	25	63·5
Ergotoxine strain (II) ..	1190	202	933	103	25	60
Ergotamine strain (I)	560	360	500	31	17	21
Ergotamine strain (II) ..	465	340	398	17	10	14
Mixed alkaloid strain ..	810	405	586	77	13	30

G. M.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline, Modification by Drugs of the Response of the Rat's Uterus to. M. Holzbauer and M. Vogt. (*Brit. J. Pharmacol.*, 1955, **10**, 186.) Twenty-seven drugs, among them the better-known adrenergic blocking agents, were tested for modification of the inhibition by adrenaline of the carbachol contractions of the non-pregnant rat's uterus. The halo-alkylamines, notably dibenzyline, sensitised the uterus to adrenaline and isoprenaline and have proved useful in biological assays of these compounds. Lysergic acid diethylamide and dihydro-ergotamine antagonized the inhibitory action of adrenaline. The effects of isoprenaline were less affected than those of adrenaline, so that this property could be made use of in distinguishing adrenaline from isoprenaline. β-Tetrahydronaphthylamine and 5-benzyloxygramine increased and Medmain slightly decreased the sensitivity to adrenaline. 5-Benzyloxygramine was the only substance encountered which sensitised more consistently to adrenaline than to isoprenaline. Veratramine in low concentrations increased the sensitivity of some uteri to adrenaline, but the action was evanescent and disappeared spontaneously or with higher concentrations of veratramine. The anti-amine-oxidases, ephedrine and iproniazid had no effect on the inhibitory action of adrenaline. G. P.

Adrenaline Stabilisation in the Adrenal Gland, a Possible Mechanism of. D. G. Humm, M. Roeder, M. Landew and E. E. Clark. (*Brit. J. Pharmacol.*, 1955, **10**, 163.) The oxidation of dihydroxyphenylalanine (DOPA) and adrenaline, estimated manometrically, was decreased on incubation with extracts of adrenal medulla homogenates. Boiling of the extract before incubation destroyed its antioxidant activity; addition of 10^{-3}M copper, cobalt or manganese to the extract also resulted in a loss of activity. Dialysis of the extract increased activity. Neither sulphydryl compounds nor ascorbic acid were responsible for the antioxidant effect and evidence pointed to a protein being involved. It was suggested that this protein, by forming a complex with adrenaline in the gland, prevents its oxidation under physiological conditions. G. P.

Antihistamine Drugs, Bronchoconstrictor and Bronchodilator Actions of. D. F. Hawkins. (*Brit. J. Pharmacol.*, 1955, **10**, 230.) Twelve well-known antihistamine drugs were found to have bronchoconstrictor action within the range of concentrations 10^{-6} to 10^{-4}, when tested on the isolated guinea-pig tracheal chain. In spinal cats and anæsthetised dogs intravenous doses within the range 1 mg. to 10 mg./kg. had similar effects. There was no correlation between antihistamine and bronchoconstrictor activities of the drugs tested. In concentrations higher than 10^{-4} the antihistamines caused relaxation of the guinea-pig tracheal chains. Mepyramine, promethazine and antazoline had similar constrictor and dilator effects on isolated human bronchial chains. The bronchoconstrictor action of mepyramine 10^{-5} on guinea-pig tracheal chains was not antagonised by atropine 10^{-5}; atropine 10^{-4} had itself a constrictor action. These direct actions of the antihistamines were suggested as being "histamine-like" and may be due, at least in part, to histamine-releasing properties of the drugs; they may be a factor limiting the therapeutic activity of the antihistamines in asthma. G. P.

Chlorpromazine, Complications of Therapy. J. Lomas, R. H. Boardman and M. Markowe. (*Lancet*, 1955, **268**, 1144.) This report is based on observation of 800 mental hospital patients treated with chlorpromazine between November, 1953, and the end of 1954. Though all types of mental cases were treated the great majority were acute or chronic psychoses. The

dosage varied considerably, but the most common procedure was to start with 150 mg. daily and to increase rapidly to 300 mg. daily; a few patients received up to 800 mg. daily. The duration of treatment varied from a few weeks to several months. The majority of patients received the chlorpromazine by mouth and were ambulant from the start. Most patients were receiving barbiturate sedation. The total number of toxic reactions recorded in the 800 patients was 59 (7·4 per cent.). Of the side-effects hypotension is the only one requiring special precautions and then only in the aged and those with cardiac dysfunction. Toxic manifestations included jaundice, blood dyscrasias, skin reactions and œdema, pyrexia, and epilpetic fits. Of these, jaundice and blood dyscrasias may prove fatal and are regarded as absolute contraindications to continuing treatment with chlorpromazine; previous liver dysfunction should also be regarded as a contraindication. Whereas the dosage at the time of the toxic reaction, the total dosage, and the method of administration, appeared of no significance, the duration of treatment before the reaction was much more constant. The greatest incidence of pyrexia was at the beginning of the second week, and both jaundice and skin reactions came on about 2 weeks later. Only in the case of epileptic fits was this time relationship not observed. Toxic reactions, except for epileptic fits, were very rare after the end of the 5th week. It is suggested that the toxic effects are due to sensitisation, and some support is lent to this suggestion by the development of eosinophilia in a few cases. The fact that the epileptic fits had no relationship either to dosage or duration of treatment suggests that chlorpromazine has epileptogenic properties which may lead to manifest fits in susceptible people. S. L. W.

Cyanacetic Acid Hydrazide, Antituberculous Activity of. M. Barnett, S. R. M. Bushby, R. Goulding, R. Knox and J. M. Robson. (*Brit. med. J.*, 1955, **2**, 647.) Cyanacetic acid hydrazide has been found to be as toxic as isoniazid and to have only one-fiftieth of the antituberculous activity of isoniazid *in vitro*. The LD 50 dose in mice was 230 mg. per kg. intravenously compared with 170 mg. per kg. for isoniazid. Strains of *Mycobacterium tuberculosis* resistant to isoniazid were generally resistant to cyanacetic acid hydrazide, but two isoniazid resistant strains were found which were sensitive to cyanacetic acid hydrazide. The lower antituberculous activity of cyanacetic acid hydrazide was confirmed *in vivo* in mice, where 2·0 mg. of isoniazid, given twice daily subcutaneously, gave complete protection; while doses of cyanacetic acid hydrazide ten times as great gave incomplete protection. By the intracorneal test in mice 0·3 mg. of cyanacetic acid hydrazide daily had no suppressive effect, and combined with 4 mg. per day of streptomycin the effect was only the same as streptomycin alone. The results suggest that isoniazid and cyanacetic acid hydrazide act in the same way. It is suggested that cyanacetic acid should not be used alone clinically and it is unlikely that there will be many instances where cyanacetic acid hydrazide will be more effective than isoniazid. G. F. S.

Dinitro-o-cresol, Potentiation of Barbiturate Anæsthesia by. E. F. Edson and F. M. Carey. (*Brit. med. J.*, 1955, **2**, 104.) A series of experiments using rats showed that, in the presence of sufficiently high dosages of dinitro-*o*-cresol to cause characteristic DNC-poisoning, 5 out of 6 barbiturates tested were significantly potentiated in rapidity of onset, depth, and duration of narcotic effects. The action of the two thiobarbiturates tested—thiopentone and thialbarbitone—was potentiated to such an extent that normally non-fatal anæsthetising doses led to almost immediate cyanosis and death. Of the four oxygen barbiturates examined, pentobarbitone, amylobarbitone, and phenobarbitone, were markedly potentiated by DNC but hexobarbitone did not

appear to be significantly influenced. The results obtained suggest that barbiturate potentiation is only likely to occur with DNC dosages high enough to cause characteristic signs of poisoning and profound disturbance of cellular oxidation-phosphorylation processes. Low dosages, causing no obvious DNC-poisoning, did not appear to increase barbiturate narcosis. The authors strongly recommend that barbiturate drugs, especially thiobarbiturates, should not be used for the induction of surgical anæsthesia in a person suffering from DNC-poisoning and that barbiturate sedation in such cases should be carried out with comparatively small doses. S. L. W.

Histamine Liberation in the Rat and Mouse. J. F. Riley and G. B. West. (*Arch. int. Pharmacodyn.*, 1955, **102**, 304.) The chronic administration of the histamine liberator, compound 48/80, to rats and mice resulted in a loss of tissue histamine and of mast cells in the ears and subcutaneous connective tissue. The drug was injected intraperitoneally once or twice daily, in doses increasing to 1 mg. daily for rats and 300 μg. daily for mice, for up to 31 days. With the first injection the rats showed visible skin œdema, shock, erythema followed by cyanosis of the ears, itching and some prostration. Under the same conditions mice showed weakness, erythema and cyanosis, but few signs of severe itching or œdema. With both species the maximum tolerated dose was used, but histamine and mast cell depletion was only moderate in mice, whereas in rats depletion was almost complete. It was concluded that histamine release plays only a small part in the action of compound 48/80 in mice. G. P.

6-Mercaptopurine in the Treatment of Leukæmia and Allied Disorders. J. R. Fountain. (*Brit. med. J.*, 1955, **1**, 1119.) This is a study of a small unselected series of patients suffering from leukæmia or allied disorders treated with a purine antagonist 6-mercaptopurine (Purinethol), an analogue of adenine and hypoxanthine. None of the patients received other forms of chemotherapy previous to, or as a supplement to, treatment with 6-mercaptopurine. The initial dose of the drug was 2·5 mg./kg. by mouth daily. The same dosage scheme was used both for patients with acute or with chronic leukæmia and in all instances the daily amount was divided into doses of 25 or 50 mg. A delay of 7 to 28 days was usual before the drug took effect. Of 13 patients with acute leukæmia 7 responded to treatment; 5 developed a complete clinical and hæmatological remission lasting from 7 weeks to 7 months, 2 showed temporary and partial improvement, and 6 failed to respond to treatment, 4 dying within the first week. Of 7 patients with chronic myeloid leukæmia a varying response was observed in 6, the other patient dying within a week of starting treatment. Subjective improvement—increase in well-being, activity, appetite and weight—was observed in 4 cases, and objective improvement—diminution in size of spleen, lymph nodes, etc.—was observed in 5 cases. The leucocyte count fell in all 6 cases. Two patients with chronic lymphatic leukæmia showed no clinical or hæmatological improvement after 1 month's treatment, and 2 patients with multiple myeloma and one with erythroderma also failed to benefit. An important feature of the drug is its low toxicity. Apart from depressing bone-marrow function no definite toxic side-effects were observed. Hæmorrhage, a possible complication, did not occur. No evidence was seen of megaloblastic erythropoiesis. The results show that 6-mercaptopurine may produce temporary remissions in some cases of acute leukæmia and modify the course of chronic myeloid leukæmia but continuous therapy seems essential if the beneficial effects are to be maintained. Although the remissions are only temporary, with the knowledge that aminopterin, amethopterin, ACTH and cortisone, and

6-mercaptopurine may all produce clinical and hæmatological remissions in acute leukæmia, symptomatic relief may be obtained by sequential use of these drugs. S. L. W.

Oxamycin, Clinical Observations on. H. J. Robinson, C. Morgan, D. W. Richard, B. M. Frost and E. Alpert. (*Antibiotic Med.*, 1955, **1**, 351.) Oxamycin is a broad-spectrum antibiotic produced by a strain of *Streptomyces garyphalus* (a comparison of oxamycin and cycloserine indicates that these products are identical). Oxamycin is a zwitter-ionic, crystalline material, somewhat unstable in acidic and neutral solutions but highly stable to prolonged treatment with base. It is active *in vitro* and *in vivo* against gram-positive and gram-negative bacteria and is efficacious against rickettsial infections and certain protozoa. It also inhibits some strains of *Mycobacterium tuberculosis* in concentrations of 5 to 10 μg./ml. but in experimental tuberculosis in mice only slight activity could be demonstrated; an *in vivo* potentiating effect of combinations of oxamycin and dihydrostreptomycin has been reported. A total of 47 patients were concerned in this study: 25 with various non-infectious diseases (in most cases cardiovascular disease) were employed for tolerance studies; the remaining 22 patients had various bacterial infections of which pneumococcal lobar pneumonia was the most common. In the first group doses of 0·2 g. twice daily were well tolerated, but when doses of 1·6 to 2·4 g. daily were given in divided doses every 6 hours toxic symptoms occurred in 11 out of 13 patients. The toxicity appeared to be confined to the central nervous system. Lethargy and somnolence were the most frequent side-effects, but other reactions included vertigo, opthalmoplegia, blurred vision and disorientation. All the effects were transient and disappeared within 1 or 2 days of withdrawing the drug. Four patients tolerated the 2·4 g./day dose level without showing toxic signs and one of them received 39·8 g. over a 25-day period. Of the 22 patients with bacterial infections 19 tolerated a daily dose of 0·8 g. of oxamycin; 3 patients became lethargic after 4 to 5 days of treatment; 2 of these subsequently became disoriented and one (with impaired renal function) developed convulsive seizures. Of the 17 cases of pneumonia oxamycin produced a good therapeutic effect in 3 cases, a moderate improvement in 6 cases, and no response in 8 cases. All of the latter cases responded dramatically to penicillin; there was some suggestion that penicillin may act synergistically with oxamycin. S. L. W.

Phenytoin Sodium, Megaloblastic Anæmia due to. G. M. S. Ryan and J. W. B. Forshaw. (*Brit. med. J.*, 1955, **2**, 242.) Three cases of megaloblastic anæmia, which were probably induced by phenytoin sodium, are described. The patients had been taking 200 or 300 mg. of phenytoin sodium, daily, in conjunction with phenobarbitone, over periods of several years. This condition is rare, and no further cases were discovered from a survey of 102 epileptic patients on phenytoin sodium therapy. The anæmia is corrected by folic acid therapy; the response to vitamin B_{12} is variable. In view of the rarity of the condition and the excellent response to treatment the use of this drug is not contraindicated, but it is probably wiser to change to another anticonvulsant drug in those patients who develop a megaloblastic anæmia. S. L. W.

Piperoxan in the Treatment of Adrenaline Overdosage. B. J. Freedman. (*Lancet*, 1955, **269**, 575.) The author reviews 11 fatal and 3 non-fatal published cases of overdosage of adrenaline and concludes that the minimum lethal subcutaneous dose for an adult is about 4 mg. and the maximum tolerated dose 7 to 8 mg. A description is given of a case in which an asthmatic was given intramuscularly 5 ml. instead of 5 minims of a 1 : 1000 solution of 80 per cent.

adrenaline and 20 per cent. noradrenaline with recovery following the intravenous injection of 10 mg. of piperoxan (2-(1-piperidylmethyl)-1 : 4-benzodioxane hydrochloride). Piperoxan is one of a number of dioxanes which are adrenergic blocking agents but it has the advantage of exerting no sympatholytic action unless given in amounts equal to 40 to 80 times the effective adrenolytic dose. In phæochromocytoma intravenous doses of 0·25 mg./kg., injected during 2 minutes, cause a fall of blood-pressure which is fully developed at the completion of the injection and may remain so for a few minutes. The blood-pressure gradually rises to its original level in 15 to 25 minutes. Side effects, such as tachycardia, headache and flushing, may occasionally occur. In accidental overdosage of adrenaline, an adult weighing 10 stones should be given intravenously without delay 15 mg. of piperoxan or 30 mg. if the dose of adrenaline was 20 mg. of more. H. T. B.

Polymyxin B and E, Release of Histamine by. S. R. M. Bushby and A. F. Green. (*Brit. J. Pharmacol.*, 1955, **10**, 215.) Polymyxins B and E injected subcutaneously in rats were about as active as compound 48/80 in releasing skin histamine remote from the site of injection, and causing degranulation of the mast cells of the mesentery. In guinea-pigs the responses to intradermal polymyxin B or E (measured by leakage of dye from the capillaries) were slightly less than with 48/80. In dogs and cats, however, the histamine liberating activity of the two antibiotics was not great, a small rise in blood histamine occurring in dogs associated with vasodepressor and bronchoconstrictor effects. The tachyphylaxis observed with repeated doses of the polymyxins was also associated with decreased histamine liberation. The doses used in the experiments approximated to lethal doses (5 to 10 mg./kg.) and toxic effects observed were severe vascular engorgement, particularly of the liver and a resulting fall in blood pressure and respiratory depression. These observations may be related to the observation that antihistamines abolish some of the side effects of polymyxin in man. G. P.

Pyrexin, Relation to some Bacterial Pyrogens. V. Menkin. (*J. Lab. clin. Med.*, 1955, **46**, 423.) The paper reports the results of an extensive investigation of the relation between bacterial pyrogens and pyrexin, the heat-stable, crystalline, pyrogenic factor isolated from the euglobulin fraction of acid inflammatory exudates. It has been suggested that its presence in exudates explains the primary mechanism of fever with acute inflammation, but as rabbits acquired a tolerance to repeated doses of 0·25 mg. of pyrexin the suggestion has been questioned. Since the pyrexin concentration in exudates varies from 1·2 to at least 9·8 mg./ml., this development of tolerance was reinvestigated using higher doses, 2 series of rabbits being given daily injection for 10 days of 0·5 ml. of saline containing 4 to 15 mg. of pyrexin. No evidence of any acquired tolerance was found either in the temperature elicited or in the duration of fever. Studies were next undertaken to determine the effect on the pyrogenic activity of the serum, of the intravenous injection into rabbits of various doses of two different bacterial pyrogens. The pyrogenic activity of the serum was found to be localised primarily in the euglobulin fraction but it was not established whether the euglobulin fraction had been converted to a pyrogenic factor or whether a pyrogen-euglobulin complex had been formed. Incubation of blood serum and a bacterial pyrogen *in vitro* resulted in equal distribution of pyrogenic properties in the euglobulin and the alpha- and beta-globulin fractions. In contrast to the bacterial pyrogens, pyrexin, when

(ABSTRACTS—*continued on page* 69)

PHARMACOPŒIAS AND FORMULARIES

ADDENDUM 1955 TO THE BRITISH PHARMACOPŒIA 1953*

REVIEWED BY G. E. FOSTER

Rapid advances in medicine and pharmacy have encouraged the General Medical Council to take advantage of that section of the Medical Act, 1950, empowering the Council to publish, between editions of the British Pharmacopœia, amendments to the current edition. To fulfil this purpose the Council has caused to be published the Addendum, 1955, to the B.P. 1953, Besides monographs on new official drugs and their preparations, the Addendum includes monographs on tablets prepared from drugs already official. Opportunity has also been taken to amend existing monographs, where necessary, in order to bring them in line with the most recent knowledge and to correct some minor errors which appeared in the 1953 edition. Of the drugs now afforded official status by inclusion in the Addendum the reviewer was impressed by the number, which had previously been described in editions of the British Pharmaceutical Codex.

The following new drugs are included in the Addendum:—

Carbimazole*
Chloroquine Phosphate*
Chloroquine Sulphate*
Corticotrophin†
Cortisone Acetate*†
Dextran Sulphate†
Diethylcarbamazine Citrate*
Ferrous Gluconate*
Gallamine Triethiodide†
Hexamethonium Tartrate*†
Insulin Zinc Suspension
Insulin Zinc Suspension (Crystalline)
Insulin Zinc Suspension (Amorphous)
Iopanoic Acid*
Isoniazid*
Lignocaine Hydrochloride†
Mersalyl Acid†
Methylamphetamine Hydrochloride*†
Nalorphine Hydrobromide†
Oxytetracycline Dihydrate*
Oxytetracycline Hydrochloride
Phenindione*
Phenylbutazone
Primidone*
Suxamethonium Chloride†

* = Tablets included; † = Injection included

Monographs are also given for tablets of Amphetamine Sulphate, Carbarsone, Di-iodohydroxyquinoline, Hyoscine Hydrobromide, Morphine Sulphate, Pentobarbitone Sodium, Quinidine Sulphate and Soluble Aspirin.

The new drugs cover a broad field of therapeutic agents, the greatest number of additions being in the hormone category. Corticotrophin (ACTH) and Cortisone Acetate both find places, while Insulin Zinc Suspension (I.Z.S.), I.Z.S. (amorphous) and I.Z.S. (crystalline) are all included. It may cause some surprise that Isophane Insulin has been omitted particularly as it has been described in the U.S.P. XV. A requirement for glycerin content has been added to the monographs on Insulin Injection, Protamine Zinc Insulin Injection and Globin Zinc Insulin Injection.

*Pp. xviii + 94. Published for the General Medical Council by the Pharmaceutical Press, London. 21s.

Of the amendments the inclusion of Purified Water to replace Distilled Water is notable and reflects the increasing use in pharmacy of demineralised water, prepared from potable water by treatment with ion exchange materials. The standard for this type of water, which may also be prepared by distillation, is the same as that previously applicable to Distilled Water with the addition of requirements for pH and albuminoid ammonia content. It will be surprising if the former requirement does not cause trouble, for it is difficult to determine the pH of an unbuffered product like distilled water and on this account one might have expected the B.P. to give specific directions for carrying out the determination. The formulæ of Syrup of Lemon, Syrup of Orange and Calamine Lotion have been amended in order to provide improved preparations.

The attention given to drugs of poor keeping properties is indicated by additional storage conditions. In the case of insulin preparations the statement that they should not be exposed to temperatures exceeding 20° C. has been deleted; the preparations are directed to be kept at as low a temperature as possible above their freezing points. Tablets of Glyceryl Trinitrate and Capsules of Halibut Liver Oil are to be labelled with their date of manufacture and these products are expected to retain their potencies for one and three years respectively when stored as directed by the B.P. The latter statement will create difficulties as pharmacists will naturally consider any stock, after storage for the period indicated, to be unfit for sale. No fault can be found with the B.P. in cases where a product is known to be unstable and an appropriate 'life' has been generally accepted, but when it is known that some manufacturers have carried out research to produce preparations of improved keeping properties it would seem fairer to require that the label should indicate a period during which the product might be expected to retain its potency and to leave each manufacturer to give a 'life' to his own product.

By describing official analytical methods and reagents a new pharmacopœia gives a good indication of advances in pharmaceutical analysis. The Addendum 1955 employs an interesting determination of zinc in the different types of Insulin Zinc Suspension and for this purpose makes use of an extraction titration using a solution of diphenylthiocarbazone in chloroform as reagent. This extraction technique has wide application and is not so well known as it deserves. In view of the established usefulness of titrations of organic bases and their salts in glacial acetic acid with standard perchloric acid solutions, it might have been expected that the Addendum would have found some use for this analytical tool. Both the B.P.C. 1954 and the U.S.P. XV use non-aqueous titrations in appropriate cases and it can only be a matter of time before the B.P. follows their example. The disintegration test for tablets, originally included in the 7th Addendum to the B.P. 1932 and official in subsequent editions of the B.P., has been replaced by a test, similar in principle to that described by Prance, Stephenson and Taylor (*Quart. J. Pharm. Pharmacol.*, 1946, **19**, 286) in a paper presented to the 1946 British Pharmaceutical Conference. There is no doubt that the new test has many advantages over the old.

The importance of biological assays is again emphasised by the inclusion of sections dealing with assays of antibiotics, serological and bacteriological products, hormones and Dextran Sulphate. The test for retardation of insulin effect for Injection of Protamine Zinc Insulin has been amended to allow guinea-pigs as well as rabbits to be used as experimental animals. The fact that it is usual to take the blood sample from the heart rather than from the ear vein in the case of guinea-pigs (Stewart and Smart, *J. Pharm. Pharmacol.*, 1953, **5**, 939) appears to have been overlooked, and it is not made sufficiently clear that the guinea-pig may also be used for the testing of I.Z.S. preparations. The test for pyrogens has been rewritten and now takes the form of a sequential sampling technique designed to use a minimum number of rabbits.

The publication of this Addendum will reassure any who may have feared that the B.P. is not keeping abreast of the most recent advances in medicine and pharmacy. The work has been well produced and proof reading has been well done the reviewer noticing no typographical errors.

The Addendum becomes official from March 1, 1956.

(ABSTRACTS—*continued from page* 66)

injected intravenously into rabbits in doses of 1·4 to 9·9 mg., did not induce pyrogenic activity in the serum. The pyrogenic euglobin of rabbit serum, produced by the intravenous injection of bacterial pyrogens, when injected intravenously into a second rabbit, also failed to induce pyrogenic activity in the recipient rabbit's serum. When injected into dogs, the pyrogenic euglobulin of rabbit serum produced a rise in temperature, and also an initial leucocytosis and a subsequent leucopænia, and the same effects were produced by the alpha-globulin from the rabbit serum. The results suggest that pyrexin from inflammatory exudates and pyrogenic euglobulin formed in the circulatory blood by interaction of bacterial pyrogens are essentially similar in nature. H. T. B.

Pyrogens in the Production of Fever. E. Atkins and W. B. Wood. (*J. exp. Med.*, 1955, **101**, 519.) The rate of clearance of intravenously injected typhoid vaccine was studied in unsensitized, sensitized and pyrogen-tolerant rabbits by means of a passive transfer technique. The blood of unsensitized rabbits which had not previously been exposed to bacterial pyrogen remained pyrogenic for normal recipients throughout a period of 2 hours following the injection. In contrast, rabbits sensitized by having received either one or two injections of the vaccine at least 3 weeks prior to the experiment cleared their blood of the test vaccine within 30 minutes despite the fact that they exhibited the same febrile response as unsensitized rabbits. After 1 hour, however, a transferable pyrogenic substance was again demonstrable in the sera of this group. It is thought that this newly appearing substance may be of endogenous origin and may be the factor which directly affects the thermoregulatory centres of the brain. Rabbits made tolerant by repeated daily injections of vaccine have a characteristically depressed febrile response. Not only were the blood streams of such animals cleared of the injected vaccine within less than 5 minutes but samples of their sera obtained 1 and 2 hours after the injection also failed to contain demonstrable quantities of the secondary pyrogen observed in sensitized animals. The latter observation is in keeping with the suggestion that the secondary pyrogen may play a critical role in the production of fever. S. L. W.

(ABSTRACTS—*continued on page* 70)

BOOK REVIEW

INTRODUCTION TO CHEMICAL PHARMACOLOGY, by R. B. Barlow. Pp. xiv + 343 including Index and 53 diagrams. Methuen & Co. Ltd., London, 1955. 35s.

The author states that the book is an outcome of the course in chemical pharmacology for students in chemistry commenced at the Department of Pharmacology at Oxford in 1945. Consequently the presentation of the material is such that students who have received little training in biological subjects are supplied with sufficient biological background to make the book intelligible. In the reviewer's opinion the author has succeeded in writing a stimulating and informative book which in certain sections exceeds the scope which the word "introduction" implies.

The book commences with a very short review of the theories of drug action. The next sections are arranged under the headings: (1) Drugs which produce general central nervous depression (such as general anæsthetics, hypnotics); (2) drugs which depress certain centres of the central nervous system (analgesics, anticonvulsants, etc.); (3) drugs which stimulate the central nervous system; (4) drugs which act on peripheral nerve endings of synapses (local anæsthetics, acetylcholine-like compounds, mydriatics, spasmolytics, ganglionic-blocking agents, neuromuscular blocking agents, anticholinesterases, adrenaline and related compounds); (5) drugs which act on tissues and organs (histamine and antihistamine drugs, drugs which act on heart muscle, etc.). In an appendix a brief account of the anatomy, physiology and biochemistry of the human body is given for the benefit of those readers who lack the necessary biological background.

The discussion of the drugs acting in a particular manner includes a brief description of the methods of testing, a detailed treatment of the type of compounds and a discussion of the attempts to correlate structure with activity.

A plentiful supply of structural formulæ and the use of tables greatly facilitates the reading of the text. It is unfortunate that a number of mistakes occur in these formulæ, e.g. p. 89 α-eucaine and β-eucaine, p. 75 camphor, p. 88 cocaine (incorrect stereochemically). Furthermore the piperidine ring of tropine (and related compounds) and ecgonine (and related structures) has been shown in the boat form in all cases, whereas there is much evidence available to show that it exists in the chair form. However, these are only minor blemishes in a well illustrated text.

This book will prove to be of great value to students reading for the B.Pharm. degree and it can also be highly recommended to all those who seek to get a background to the structure and action of many of the newer type drugs. The research student embarking upon the preparation of potential pharmacologically-active compounds will also derive much benefit from this introduction to the subject.

A. H. BECKETT.

(ABSTRACTS—*continued from page* 69)

Reserpine, Serotonin and Lysergic Acid Diethylamide, Interaction of in Brain. P. A. Shore, S. L. Silver and B. B. Brodie. (*Science*, 1955, **122**, 284.) Experiments in mice show that reserpine potentiates the hypnotic effects of hexobarbitone and ethanol, and lysergic acid diethylamide antagonises the potentiation. Lysergic acid diethylamide alone did not affect the hypnotic action of hexobarbitone or ethanol. The results suggest that some of the actions

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LETTER TO THE EDITOR

The Assay of Artemisia

SIR,—We are grateful for the opportunity, with the permission of the author, to read in proof the paper by Qazilbash on the assay of artemisia. This we have done with considerable interest, well knowing of his wide experience in this subject. Ours, we are well aware, is far more limited, nevertheless we ask for your courtesy to publish the following comments.

1. The purpose for which our assay process was worked out is quite different from that for which Qazilbash carries out his analyses, hence we are not bound to share quite the same views.

2. Qazilbash makes many criticisms of our process and states in his conclusion that the results obtained by his revised method are "better qualitatively as well as quantitatively", but he gives no indication in his paper by published figures that he has carried out even one assay according to our process.

3. The quantity of barium hydroxide used by us is not really in any larger excess than that used by him. For approximately 80 to 200 mg. santonin he uses 110 ml. freshly prepared 5 per cent. solution; for about 280 to 500 mg. we use 250 ml. of a saturated solution (prepared by dissolving barium hydroxide in boiling carbon-dioxide-free water, allowing to cool overnight without access of air, and filtering immediately before use); the strength is approximately 6·5 per cent.

4. Qazilbash does not give any evidence to show that our use of chloroform with the baryta gives a less pure yield of santonin, although in para. (iv) p. 28 he makes this statement.

5. The chief differences in our respective methods are in the weight of the yield of santonin and the correction used. Qazilbash, apparently, usually weighs 30 to 150 mg., we aim to obtain 230 to 450 mg. The corrections in both methods are not dissimilar, but the ratio correction/yield is far larger in the case of his process. As far as the correction itself is concerned, we would say that, much to our regret, we have been unable to find time to carry out any further work in this direction and we are much interested in the figures published in Qazilbash's paper. As we deal with two-and-a-half to three times as much yield for the use of less than twice as much solvent, our final filtration is necessarily quite different from his, and we would here reiterate that we did not, for this particular material, find kieselguhr helpful or necessary, while we would not wish to detract from its value for many other types of filtration.

6. Regarding the purity of the yield, as determined by its melting point, we would point out that we expect to obtain melting points of 173° C. or higher by our published method. Qazilbash, in Table I, only gives six figures out of a total of twenty-six as high as this, and of these six, two were obtained by the use of animal charcoal alone.

7. Qazilbash, in 1952, stated that his then published method was unsuitable for low-grade material. In the conclusion to his present paper it appears that his revised method can be modified in exactly the same manner as that suggested by ourselves for our process; we would stress, however, that we work always on larger weights of material.

The three of us who co-operated in the publication of our process are now separated, and, much to our regret, it seems unlikely that we shall be able to

carry out further work on the assay of artemisia. We thank you for allowing us to publish this note.

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October 13, 1955.

(ABSTRACTS—*continued from page* 70)

of reserpine may be mediated through the release of serotonin in the body. Experiments in dogs, which show a high increase in the excretion of 5-hydroxyindoleacetic acid, a major metabolite of serotonin, support this hypothesis.

G. F. S.

Silicone Aerosols, Control of Pulmonary Œdema. M. Nickerson and C. F. Curry. (*J. Pharmacol.*, 1955, **114**, 138.) Experimental pulmonary œdema, induced in rabbits by intravenous injection of adrenaline and in rats by inhalation of chlorine, was effectively controlled by the anti-foaming action of inhaled aerosols of dimethylpolysiloxane emulsions. In preliminary *in vitro* experiments with a 10 per cent. serum solution the most effective and easily handled silicone emulsion was XEC 151, (30,000 cs. dimethylpolysiloxane with 5 per cent. SiO_2), foaming being prevented where the silicone concentration was as low as 6×10^{-9}. A 10 per cent. aqueous emulsion of XEC 151 gave complete protection in rabbits against intravenous doses of adrenaline of 1 mg./kg. or less and 60 per cent. protection against 2 mg./kg. Emulsion XEF 215 (500 cs. dimethylpolysiloxane) was almost as effective as XEC 151. Death from chlorine-induced pulmonary œdema in rats was also prevented by these aerosols, but the range of exposure within which protection was obtained was much narrower than with adrenaline. It was suggested that the limits of dosage of adrenaline or chlorine against which the aerosols can protect are determined by toxic actions other than the production of pulmonary œdema. Inhalation of the aerosols did not alter oxygen transfer in the lungs of dogs and daily administration for up to 38 days did not produce inflammatory or granulomatous changes in the lungs of rats. Other workers have reported the low toxicity of these aerosols after both acute and chronic administration by various routes.

G. P.

Steroid Anæsthetic Agent. G. D. Laubach, S. Y. P'an and H. W. Rudel. (*Science*, 1955, **122**, 78.) The anæsthetic activity of a number of water-soluble steroids was compared with thiopentone sodium. The most promising of the series was hydroxydione (21-hydroxypregnane-3: 20-dione sodium succinate), which in mice and rats had intravenous anæsthetic potency equal to that of thiopentone sodium, but a much higher therapeutic index. In cats, dogs and monkeys the therapeutic index of the steroid was again high, but anæsthetic potency was only one-fourth of that of the thiobarbiturate. Respiratory depression during hydroxydione anæsthesia was relatively low and recovery rapid, uncomplicated and with minimum post-anæsthetic depression. Even with large doses of the steroid little or no endocrine activity was observed. G. P.